FIELD ECOLOGY AND IMPACT OF THE SEED-FEEDING BEETLE *ACANTHOSCELIDES MACROPHTHALMUS*, A BIOLOGICAL CONTROL AGENT OF THE INVASIVE TREE *LEUCAENA LEUCOCEPHALA*, IN THE KWAZULU-NATAL COASTAL REGION

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PREFACE

The research described in this dissertation was carried out in the School of Life Sciences (Pietermaritzburg campus) from February 2011 to June 2014 under the supervision of Dr T. Olckers.

The work presented in this dissertation represents the original work of the author and has not been otherwise submitted in any other form for any degree or diploma to any other University. Where use has been made of the work of others, this has been duly acknowledged in the text.

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ABSTRACT

Introduced for agroforestry, the Mexican tree *Leucaena leucocephala* (Fabaceae) has become invasive in several tropical and subtropical regions worldwide. In South Africa, the most notable infestations are located in the KwaZulu-Natal (KZN) coastal region. A seed-feeding beetle, *Acanthoscelides macrophthalmus*, originally imported from Mexico, was released in South Africa to control the plant's excessive seed production and has become widely established in the KZN coastal region. By sampling plant populations monthly at selected field sites in this region, this study was intended to determine the: (i) seasonal (monthly) abundance of the beetle populations; (ii) levels of seed damage inflicted in relation to seed production by the plants; (iii) extent to which the beetle has recruited native parasitoids; (iv) incidence of non-target effects; and (v) ability of the beetle to regulate/control plant populations or limit their spread. Beetle numbers fluctuated greatly between months and between sites, resulting in erratic levels of seed damage ranging from 2-60%. Although ripe pods were available to the beetles throughout the year at one of the four study sites, this was not the case at the other three sites where ripe pods were virtually absent from November to January. High numbers of undamaged seeds found on the soil surface indicated the extent to which the seeds escape beetle predation. Parasitism of the beetle's larval/pupal stages by native parasitoids was variable and relatively high (up to 40%). Ten species of parasitic wasps were reared from beetle-infested seeds, the most important of which originated from native *Acacia* plants. There were no instances of non-target effects involving the seeds of native *Acacia* species. There was a strong positive relationship between wasp numbers and beetle-infested seeds, indicating that the relationship is not incidental, and that the beetle has been adopted by the wasps as a new host. The relationship between the percentage of seeds damaged by *A. macrophthalmus* and seed availability was inversely density-dependent, with higher rates of seed damage occurring when fewer seeds were available. This negative relationship between seed damage and seed availability, as well as the relatively low levels of seed damage recorded, suggest that the beetle's impact is negligible. The addition of other seed-feeding or seed-reducing agents to the *L. leucocephala* system may result in a more significant contribution from *A. macrophthalmus*.

Key words: Seed predation; seed availability; weed biological control; leucaena; parasitoids

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CHAPTER 1: Introduction

1.1 Invasive alien plants and biological control

1.1.1. General statement

The effects of invasive alien plants are evident in both natural and agricultural environments. Many alien plants that were deliberately introduced in South Africa, but also worldwide, were used for forestry, agroforestry, horticulture, fruit and stabilizing sand dunes (Zimmermann & Neser 1999). In the absence of natural enemies, such species are able to outcompete and displace natural vegetation, often with knock-on effects on both vertebrates and invertebrates that make use of native plants (van Driesche *et al.* 2010, van Wilgen & De Lange 2011). As most invasive trees are major water users, rivers and other water resources are at risk, both from high rates of transpiration as well as blockage by the trees (van Driesche *et al.* 2010). Through eutrophication, invasive aquatic plants cover the surfaces of water bodies, causing a number of problems that include reduced water quality and alteration of the ecology of the water body (van Wilgen & De Lange 2011). Amongst other negative impacts, terrestrial invasive plants promote soil erosion and the draining of soil nutrients. They not only replace grazing plants, but also grow in dense thickets that can prevent access of sheep and cattle to grazing, directly affecting agriculture (Klein 2011). Features of invasive plants include allelopathy, quick growth, dispersal by native vertebrates and high seed production (Charudattan 2005, van Driesche *et al.* 2010). In the United States of America, the cost of controlling the approximately 25 000 invasive plant species (including crop and pasture weeds), which result in annual agricultural losses of USD$24 billion, was estimated at USD$10 billion annually (Pimentel *et al.* 2000).

South Africa's water resources, agriculture and biodiversity are similarly at risk from invasive alien plants (van Wilgen *et al.* 2012). Riverine habitats have a high number of invaders, and water usage by invasive plants exceeds that of native plants by 3.3 billion m^3 (Holmes *et al.* 2005, van Wilgen & De Lange 2011). De Lange & van Wilgen (2010) calculated that annual losses from invasive plants totalled as much as R6.5 billion when considering their impacts on water resources, grazing and biodiversity, with several other studies conducted since 1996 concluding that there would be a nett monetary gain from clearing current infestations and preventing their spread. The cost of controlling invasive alien plants in South Africa

using available control methods was estimated at R3.2 billion between 1995 and 2008 (van Wilgen *et al.* 2012).

The most commonly used control methods are mechanical and chemical control, which while effective in the short term, are not always effective long-term options because of costs and their interruption of biological control operations (Gardener & Davis 1982, Zimmermann & Neser 1999). Mechanical control without herbicidal treatment is often also problematic because it requires the removal of entire plants to prevent regrowth, while disturbance of the soil during clearing operations often induces germination from the seed bank (Coetzer & Neser 1999, Olckers 2011). Chemical control methods can be problematic if the plant invades riverine areas, as there is the risk of run-off of chemicals into water systems, while herbicides fall short against plants with high seed production, because the seeds escape control when falling onto the soil (Coetzer & Neser 1999, Olckers 2011). Other control methods that are practiced but are largely ineffective include changes in land management uses such as burning, grazing by cattle and altering available nutrients (van Driesche *et al.* 2010). Where mechanical and chemical control methods are ineffective or costly, biological control becomes an attractive long-term option.

1.1.2. Biological control

Biological control programmes depend on natural enemies (agents) that comprise mostly herbivorous insects but also plant pathogens. These programmes involve several components that include locating and introducing adequate agents, confirming their host specificity (i.e. safety), releasing and establishing them in the invaded country and conducting post-release evaluations to determine their effectiveness (McEvoy & Coombs 1999). Suitable agents must inflict noticeable damage to the target plant in its native habitat, be host specific and demonstrate an ability to cause sufficient damage to the plants in their introduced habitat, including a decrease in plant reproduction and growth, and an increase in mortality (Waloff & Richards 1977, McEvoy & Coombs 1999). In relation to other control methods, biological control aims to increase water resources, reduce soil erosion and fire hazards as well as control the threat to biodiversity while remaining cost effective and environmentally friendly (Batra 1982, Le Maitre *et al.* 2002).

However, biological control is not without its shortfalls. Agent selection procedures and host-specificity testing render biocontrol time consuming and ensure that it can be expensive in the long term (Markin *et al.* 1992). Control agents are not always successful, and complete control of the target plant is seldom achieved, often as a result of slow responses of the agent(s) to invasive plants (Mack & Lonsdale 2002). Various factors that negatively affect the agents include their inability to adapt to a new climate, the misuse of chemical and mechanical control methods that disrupt their populations, the recruitment of native predators and parasitoids that attack them, as well as resistance to the agents by plant defences (Newman *et al.* 1998). Because some invasive plants have economic importance, either as forestry or fodder crops or as ornamentals, biological control efforts against such plants are often met with resistance from the public, although parties often agree that plants need to be controlled if they become invasive (Zimmermann & Neser 1999, Stanley & Fowler 2004, van Driesche *et al.* 2010). Also, concerns about the safety of biological control revolve around our inability to control agents once they are released and become widespread, and their ability to disperse and reproduce independently. Coupled with this were concerns over attacks on non-target plants, with a few highly-publicized cases (Simberloff & Stiling 1996, Taylor *et al.* 2007, Barratt *et al.* 2010). As a result, host-specific agent species and extensive post-release evaluations are essential for successful biological control programmes (Barratt *et al.* 2010).

South Africa's biological control efforts began in 1913 when *Opuntia monacantha* Haw. (Cactacae) was brought under control by *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae) (Klein *et al.* 2011). Since then, a total of 106 agent species have been released, while 64 were rejected after failing to meet the strict requirements set out by the Department of Environmental Affairs and the Department of Agriculture, Forestry and Fisheries (Klein 2011). Of those released, 57 agent species inflicted extensive or considerable damage to the target weeds, bringing 10 plants under complete control (i.e. no other control efforts required) and 18 under substantial control (i.e. other control efforts still needed but at reduced levels). Only 13 agent species inflicted trivial amounts of damage to their target plants (Klein 2011). The 'Working for Water' Programme, which was started in South Africa in 1995, is aimed at increasing water supplies, as well as providing jobs, by

means of the removal of problematic invasive plants from invaded catchments and other water resources (Zimmermann & Neser 1999). Biological control is seen as an important tool for the 'Working for Water' Programme, as it reduces the weeds' ability to re-invade areas that have already been cleared (Zimmermann & Neser 1999).The use of biological control is encouraged, not only for well-established weeds, but also "emerging" weeds that have the potential to become invasive later (Olckers 2004). The subject of this study, *Leucaena leucocephala* (Lam.) de Wit (Fabaceae), represents one of these "emerging" weeds.

1.2. *Leucaena leucocephala*

1.2.1. Description

Leucaena leucocephala (Lam.) de Wit, commonly known as leucaena, is a shrub or small tree of the family Fabaceae and is native to Central America (Parrotta 2002, Hughes 2006, Orwa *et al*. 2009). It is also known by the synonyms *Acacia leucocephala* (Lamarck) Link, *Leucaena glabrata* Rose (now *Leucaena leucocephala* subsp. *glabrata*), *Leucaena glauca* (L.) Benth., and *Mimosa leucocephala* Lamarck (now *Leucaena leucocephala* subsp. *leucocephala*) (Hughes 2006, USDA 2014). There are 23 species within the genus *Leucaena*, which is thought to be an interbreeding complex, since some of its species are capable of producing hybrids (Shelton & Brewbaker 1994, USDA 2014).

There are three known subspecies of *Leucaena leucocephala*; namely *leucocephala*, *glabrata* (Rose) Zarate and *ixtahuacana* C.E. Hughes (Hughes 2006). Two of these, *leucocephala* (Hawaiian shrubby type) and *glabrata* (Salvador tree type), were deliberately introduced to other tropical countries around the world with the former being the most widespread and invasive (Hughes 2006). The shrubby subspecies *leucocephala* reaches a height of 4m, while the subspecies *glabrata*, which is typically a woody tree, can reach 15m and lives for 20-40 years (Hughes 2006, Orwa *et al.* 2009).

The subspecies *leucocephala* is a thornless, highly branched deciduous shrub or small tree with a deep taproot (Orwa *et al*. 2009). When it forms thickets, the trees are slender with tufted crowns; however, if grown individually, trees have flat, spreading crowns (Duke

1983). Bark on young trees is a light grey-brown, turning darker as the trees mature (Orwa *et al*. 2009). The plant's dark green, glabrous leaflets are linear-oblong shaped and are acute at the tip and rounded to obtuse at the base. They are 2.5-4mm wide and 9-16mm long, with 13-21 pairs of intermediately spaced, sessile leaflets per pinna. There are usually 4-9 pairs of pinnae per leaf and leaves are 70-150mm long and bipinnate (Hughes 2006, Orwa *et al*. 2009).

White to cream-coloured flowers (Fig. 1.1a) that are 12-21mm in diameter form in dense clusters of 100-180 per flower head, with flower heads aggregating in groups of 2-6, and are most common from July to March in tropical areas (Orwa *et al*. 2009). From these flowers, the tree then produces 5-20 green pods (Fig. 1.1d) per flower head, which take 10-15 weeks to ripen and turn brown. Ripe pods (Fig. 1.1e) are 110-180mm long and contain 10-20 seeds each (Hughes 2006, Orwa *et al*. 2009). Leucaena seeds are tear-shaped, 7-10mm long and 4-6mm wide and have hard, dark brown coats (Shelton & Brewbaker 1994, Orwa *et al.* 2009). The subspecies *leucocephala* is capable of producing up to 30 000 seeds per plant (Raghu *et al*. 2005). All reproductive stages (i.e. flowers and pods) are available on individual trees throughout the year (Orwa *et al*. 2009; see Fig. 1.1). The latter two features play a major role in the plant's invasiveness.

Figure 1.1. Reproductive stages of *Leucaena leucocephala* in the field, showing the flowers in their pre-flowering (a), flowering (b) and post-flowering (c) stages and the pods in their green (d), ripe (e) and dehisced (f) stages.

1.2.2. Biology and ecology

Leucaena is a tropical plant and, as a result, its optimal growth is in tropical or subtropical climates. In its native range, it receives 750-1800mm of rain per year, with a 3-6 month dry season and grows at altitudes of up to 500m in the case of subsp. *leucocephala* and 1500m in the case of subsp. *glabrata* (Binggeli 1997). In its introduced range, populations are capable of surviving wide variations in rainfall, with some populations getting as little as 500mm per year and being subjected to 8-month drought periods, and others tolerating 3500mm per year (Brewbaker 1987, Shelton & Brewbaker 1994, Shelton & Jones 1995). Despite its wide tolerance to water availability, leucaena does not cope well with colder weather, preferring average daily temperatures of 25-30°C, with suboptimal temperatures resulting in slow growth, and growth being inhibited at temperatures below 15°C (Cook *et*

al. 2005, DAFF 2011). Because of its temperature requirements, leucaena's growth is limited to an altitude of 1000m in the tropics and 500m in the subtropics (Shelton & Brewbaker 1994).

The plant is capable of growing in various soil types, although optimal growth occurs in soils that are well drained with a slightly alkaline to neutral pH; however, it can tolerate a slightly acidic soil of pH 5.0 (Shelton & Jones 1995, Parrotta 2002). Clay soils are best for subsp. *glabrata*, while subsp. *leucocephala* prefers limestone soils (Walton 2003). Soil conditions which impede the growth of leucaena include waterlogging, acidity, high salinity and Aluminium content and low Phosphorous and Calcium content (Brewbaker 1987). The plant does not grow as effectively in the acidic soils that dominate Asia and South America (Shelton & Jones 1995).

Mature trees can cope with waterlogging and drought, by virtue of a deep root system that extends up to 5m deep (Shelton & Brewbaker 1994). A lack of water, high temperatures and frost result in the trees dropping and folding their leaflets and setting less seed (Shelton and Brewbaker 1994, Hughes 2006, Orwa *et al*. 2009). Plants resprout readily after cutting and burning (Brewbaker 1987, Walton 2003).

Leucaena generally produces flowers after two years, and flowers continually throughout the year. Flowers attract a range of generalist pollinators including bees, but are also self-compatible, and most pod set is the result of self-pollination (Brewbaker 1987, Hughes 2006, Orwa *et al.* 2009). The pods are dehiscent, with pods splitting intermittently to release their seeds onto the ground below the parent plants. Due to their hard coats, seeds are capable of surviving in the soil for 20 years before germinating (Hughes 2007). As a result, seeds require scarification to germinate, with germination rates ranging from 5-90% (Binggeli 1997; Suttie 2005). Seeds on their own are mostly not capable of dispersing for long distances, and typically do not fall more than 20m from the parent plant (Walton 2003). Leucaena seeds can, however, be spread by wind and water, and seed-eating animals such as birds, rodents and cattle, but are mostly spread by humans for cultivation (Smith 1985, Walton 2003). Despite the plant's overall quick growth, seedlings have relatively slower growth rates and are easily killed by frost or waterlogging, which is thought to be why seedlings are such poor competitors (Cooksley 1987, Shelton & Jones 1995).

1.2.3. Uses

Leucaena leucocephala is the most widely planted species within its genus (Shelton & Jones 1995). Its many uses stem from its ability to fix nitrogen, its high nutritional value for livestock and its quick growth, especially in tropical and subtropical climates (Shelton & Brewbaker 1994, Hughes 2006). It is primarily grown for cattle fodder, and is both highly palatable and extremely nutritious to livestock. Cattle gain weight rapidly when fed leucaena, gaining 0.7 - 1.7 kg per day on leucaena-supplemented diets (Walton 2003). Pods are toxic to livestock if they form too much of the animal's diet; however, a ruminant microbe, which occurs naturally in cattle in Central America, circumvents the problem of toxicity (Shelton & Brewbaker 1994).

Because of its ability to fix nitrogen, the plant is widely used in agroforestry for a variety of crop plants that are grown in the tropics, but also to provide protection from both wind and sun to crops such as coffee, tea, cacao and teak (Shelton & Brewbaker 1994). Wood from leucaena trees can also be used for both firewood and wood products, such as flooring and pulp for paper (Shelton & Brewbaker 1994, AGIS 2007). Leucaena can also be used for windbreaks and firebreaks, biofuel, preventing soil erosion and reforestation (Shelton & Brewbaker 1994, Hughes 2006). It was widely used by the indigenous people of Central America for mainly food, but also for soil restoration and green manure (Brewbaker 1987).

Cultivated leucaena has low genetic diversity, due to the seed stock taken from Central America originating from a single tree of the subspecies *leucocephala* (Shelton & Jones 1995). However, by crossing *L. leucocephala* with other species in the genus, farmers can produce high-yielding, low-seeding varieties that have less potential for weediness (Brewbaker 1987). Although many species within the genus *Leucaena* are highly disease resistant, there are some diseases to which the subsp. *leucocephala* is particularly susceptible (Brewbaker 1987). Boa & Lenné (1995) listed several diseases affecting *L. leucocephala*, with *Camptomeris leucaenae* (F. Stevens & Dalbey) Syd. (Pezizomycotina) leaf spot and gummosis being the most serious, as well as stem, root and pod rot, all caused by a variety of pathogens, mainly species of *Fusarium* Link (Nectriaceae).

There are a range of insects that attack leucaena in cultivation, including moths, ants, termites, several beetles (including seed beetles), scale insects, mealy bugs and inchworms.

Notable pests include the beetle *Araecerus fisciculatus* Degeer (Coleoptera: Anthribidae) and the moth *Ithome lassula* Hodges (Lepidoptera: Cosmopterigidae), which attack the plant's reproductive structures and have resulted in losses for farmers (Shelton & Jones 1995). However, the most prolific insect pest is the leucaena psyllid, *Heteropsylla cubana* Crawford (Hemiptera: Psyllidae). It was accidentally transported out of Central America, and spread to leucaena populations throughout the world during the 1980s (Brewbaker 1987, Walton 2003). The psyllid is the most widespread and damaging of all of the insect pests, with outbreaks causing extensive defoliation in the short term, and reduced growth and vigour over the long term (Bray & Woodroffe 1991). Losses of up to 52% have been recorded for farmers in Australia, with $2.8 million worth of losses in Java and decreased production in other South-East Asian countries (Bray & Woodroffe 1991, Shelton & Brewbaker 1994, Binggeli 1997).

1.2.4. Native and invaded range

Leucaena leucocephala is native to the Yucatan Peninsula of Central America (NAS 1977, Shelton & Brewbaker 1994). The subspecies *ixtahuacana* and *glabrata* both occur in southern Mexico and Gautemala, although *glabrata* is more widely distributed than *ixtahuacana* (NAS 1977, CABI 2014). The subspecies *leucocephala* occurs along the East and West coasts of Central America, as well as southern Mexico, and in Guatemala, Belize, Honduras and El Salvador, although it is unclear as to how much of this is its true native range due to widespread use and distribution of the plant by indigenous people throughout these areas (NAS 1977, Parrotta 2002, CABI 2014).

The initial spread of the plant outside of Central America commenced when the Spanish transported it to their colony in the Philippines in the 1600s, because of its uses as fodder and food (Brewbaker 1987, Parrotta 2002). After witnessing its versatility, the plant was transported to other colonies in South East Asia, Hawaii, Australia, India and Africa during the 1800s (NAS 1977, Binggeli 1997, Walton 2003). It is now naturalized in nearly 160 countries and territories around the world, including both North and South America, Asia, Africa, Australia and many of the Pacific Islands (CABI 2014).

1.2.5. Introduction to South Africa

Leucaena was introduced into South Africa before the 1920s, for agroforestry (Tribe 1995, Neser & Klein 1998). Because of its environmental limitations and poor seedling competitiveness, the plant was not considered a threat until it was found invading disturbed areas (Tribe 1995). Leucaena is thus considered to be an "emerging" weed in South Africa, with the potential for further spread (Henderson 2007). Most of the occurrences of the weed are in the eastern half of the country where environmental conditions are more suitable. These include a number of infestations along the KwaZulu-Natal coast, with some in the Eastern Cape, Mpumalanga, Limpopo and Gauteng provinces (Fig. 1.2).

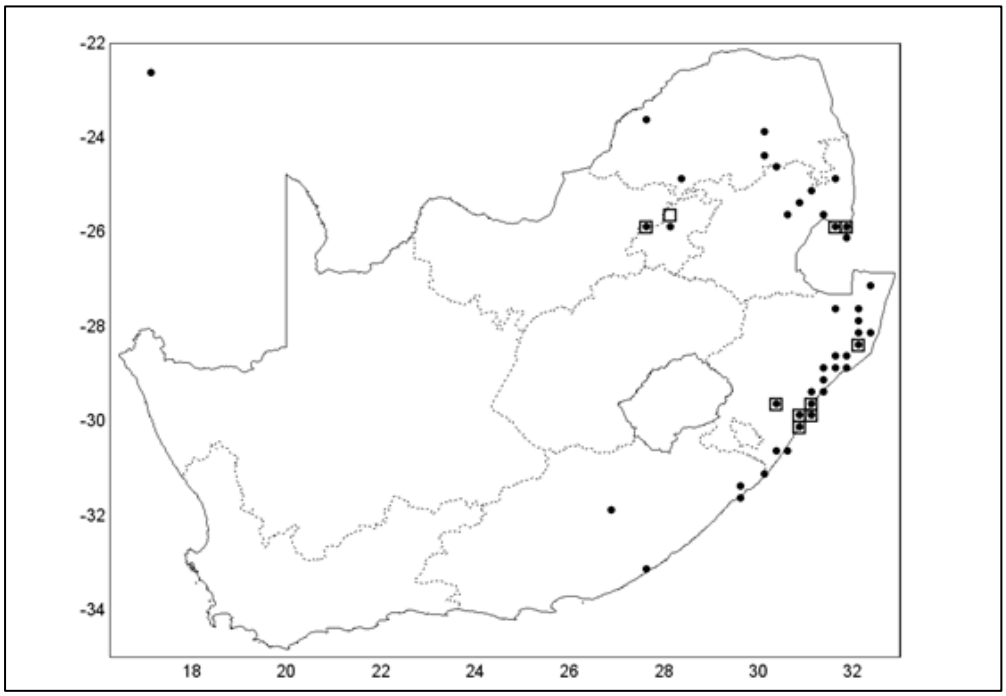

Figure 1.2. Distribution of *Leucaena leucocephala* (●) and the seed-feeding beetle *Acanthoscelides macrophthalmus* (□) in South Africa (from Olckers 2011).

Populations have been found in savannah, forest, grassland and wetland habitats (Henderson 2007). According to the Conservation of Agricultural Resources Act of South Africa, leucaena is a Category 1 invader in the Western Cape, and a Category 2 invader in the rest of the country. A Category 1 rating requires that it be removed immediately where present while a Category 2 rating stipulates that it can be grown in demarcated areas if a suitable permit is obtained (AGIS 2007). The recent National Environmental Management:

Biodiversity Act (Government Gazette, 1 August 2014, No. 37886) also lists the plant as a Category 2 invader.

1.2.6. Invasiveness and harmful impacts

Leucaena appears on the Global Invasive Species Database as one of the world's worst invaders, and is listed as invasive in almost 70 countries (Hughes 2006, CABI 2014). It is even considered weedy in its native Central America, in areas that are not part of its natural distribution (CABI 2014).

Where environmental conditions are adequate, the plant invades disturbed areas such as roadsides, but also undisturbed areas such as riparian zones and coastlands (Walton 2003, AGIS 2007). It can also occur in grasslands and agricultural lands and both natural and planted forest, although it does not appear to be invasive in these areas (Hughes 2006). When unchecked, weedy outbreaks have been reported near cultivated leucaena crops (Walton 2003).

Once they have invaded, populations are able to replace native vegetation through allelopathy, restrict access to people and livestock by growing in dense clusters, and decrease water flow when invading watercourses (Chou & Kou 1986, Bingelli 1997, Neser & Klein 1998). Researchers in Japan studied the effects of leucaena invasions on abandoned fields on the Ogasawara Islands. They found that not only did plots with leucaena have more species of other alien plants, but that they also supported a lower basal area of native plants (Yoshida & Oka 2004).

The main causes of weediness in *L. leucocephala* overlap with the plant's beneficial attributes, namely its ability to fix nitrogen, quick growth, vigorous coppicing, and high seed production due to self-compatibility and continuous flowering (Cook *et al*. 2005, CABI 2014). High seed production is a characteristic that leucaena has in common with other problematic leguminous invasive plants in South Africa (Olckers 2011). The biggest threat though is the soil seed bank, from which seeds are capable of germinating after 20 years (Hughes 2006). Because of this, risk assessments conducted in both Australia and Hawaii recommended that leucaena should not be planted (Hughes 2006).

1.2.7. Control methods including biological control

Several integrated control methods have been used to manage weedy outbreaks of leucaena. Chemical control, mechanical control and burning are the most commonly used methods, though farm management and biological control have also been employed. Controlling leucaena outbreaks in Queensland has an estimated overall cost of up to AUS$14 million per year (Walton 2003).

A variety of herbicides have been registered for use on leucaena in Australia (Walton 2003). These include foliar sprays of Roundup™, Grazon™, and Lontrel™; basal bark applications of Garlon™, Access™ and Starane™ and stem-injections of Tordon™. Diesel can also be effectively applied to both seedlings and cut stumps of leucaena without the addition of any herbicide (Walton 2003). However, none of the above-mentioned herbicides that are available in South Africa has been registered for use against leucaena (Dow AgroSciences 2014). Although these herbicides kill the plants, populations resurge readily due to the extensive seed banks, which are unaffected by herbicides (Walton 2003, DAFF 2011).

For mechanical control to be effective, removal of the roots is essential and can be achieved by mulching the plants, ploughing, or winching the plants out of the ground (Walton 2003). However, these tactics are disruptive and inappropriate for invasions in natural areas (e.g. riparian zones). Because seedlings are such poor competitors, the planting of fast-growing plants in place of leucaena can prevent population regrowth in cleared areas (Walton 2003).

Fire can be used to control leucaena, and is especially effective against seedlings (Walton 2003). However, fires need to be hot enough to prevent coppicing, which mature trees do easily after a cool fire. There is also a high rate of germination after fires, which possibly provide the scarification that the seeds require. As a result, a single burn is considered insufficient to control the plant, and other methods, such as chemical control or a second burn are required for more effective control (Walton 2003).

Farmers, especially in Australia, are encouraged to manage their leucaena fodder crops to prevent invasions in environmentally sensitive areas (Walton 2003, Hughes 2006, DAFF 2011). Intense grazing of leucaena by cattle is considered to be a method of controlling the

plant and preventing it from becoming a weed (Smith 1985). Cattle utilize both leaves and pods on trees up to 1.7m high, reducing plant growth and curtailing the addition of seeds to the seed bank (Walton 2003). Leucaena's weediness is less prevalent in areas where cattle graze or where it is cultivated for other uses (Brewbaker 1987, Shelton & Jones 1995).

Although it is listed as invasive in almost 70 countries, no countries other than South Africa have initiated biological control programmes against the plant (Olckers 2011, CABI 2014). The leucaena psyllid was considered as a possible biological control agent in Hawaii; however, this was never pursued due to concerns of farmers on the potential effects of the psyllid on cultivated leucaena (Smith 1985). Agents that attack the plant's reproductive structures are preferred, as they have the potential to curb the plant's invasiveness while still retaining its many benefits (Neser & Klein 1998). A seed-attacking beetle, *Araecerus levipennis* Jordan (Coleoptera: Curculionidae), an inflorescence-attacking moth, *Ithome lassula* Hodges (Lepidoptera: Cosmopterigidae), and a seedling-attacking fungus of the genus *Pythium* were considered to have potential for biological control in areas where the plant is cultivated for its leaves only (Shelton & Brewbaker 1994, Walton 2003). However, given the opportunistic nature of the South African biocontrol programme and the fact that a thorough assessment of all potential biocontrol agents was never undertaken, the seed-feeding beetle *Acanthoscelides macrophthalmus* (see below) is currently the only agent that has been actively deployed (Olckers 2011). The leucaena psyllid was inadvertently introduced into South Africa, having entered from neighbouring countries to the north, but its impact on leucaena populations is considered to be negligible (Olckers 2011).

1.3. *Acanthoscelides macrophthalmus*

1.3.1. Description and biology

Acanthoscelides macrophthalmus (Schaeffer) (Coleoptera: Chrysomelidae: Bruchinae; previously Bruchidae) is an endophagous seed-feeding beetle that is associated with *L. leucocephala* in its native Central America (Kingsolver 2004). The adults are small, with a body length of 2.9–3.8 mm and width of 1.8–2.0 mm. The beetle has a red integument, with grey, gold and brown setae patterned on the elytra (Fig. 1.3.) which extend three quarters

of the way down the abdomen. Adults have prominent eyes and serrate antennae (Kingsolver 2004).

Females lay eggs throughout the year, and are capable of laying around 62 eggs in their lifetime (Effowe *et al*. 2010). They lay their eggs on ripe leucaena pods, that are either suspended in the canopy or dislodged onto the ground, as well as on dehisced seeds and do not require either food or water before laying eggs (Raghu *et al*. 2005, Tuda *et al*. 2009). Eggs hatch on the surface of the seed or pod after around 5 days, and the larvae burrow through the seed coat and into the seed (Effowe *et al*. 2010). As they develop, the larvae consume the endosperm, depriving the seeds of the nutrients that are required for germination (Neser & Klein 1998). As *L. leucocephala* seeds are small, they usually support the development of one beetle, occasionally two, although up to three have been recorded (Neser & Klein 1998, Shoba & Olckers 2010). There are four larval stages which all develop in the seed. A circular hole is made in the seed coat by the final larval instar through which the adult beetle emerges after pupation. The total time for development from egg to adult takes around 34-36 days, depending on temperature (Effowe *et al.* 2010, Shoba & Olckers 2010). After emergence, the adults live for 2-20 days in the absence of nutrient and water supplements, with females laying eggs after 2-4 days (Shoba & Olckers 2010).

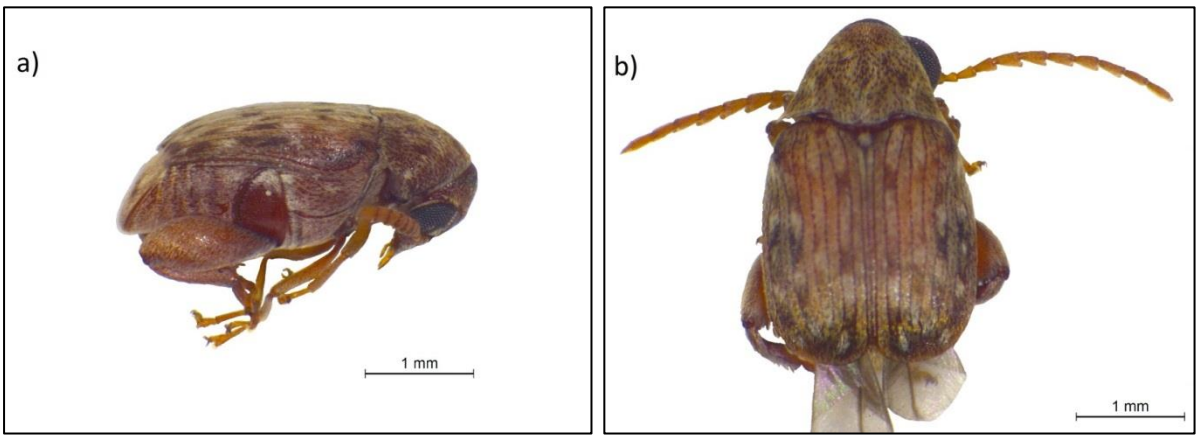

Fig 1.3. a) Lateral and b) dorsal views of adult *A. macrophthalmus* beetles.

1.3.2. Secondary distribution

The beetle was first released in South Africa in 1999, following host-specificity tests that had been conducted since 1989, when a quarantine colony was first brought over from Mexico (Neser & Klein 1998, Olckers 2004). The delay in the release of the beetle was largely due to conflicts of interest with agroforestry and other agencies that utilize the plant (Neser & Klein 1998). The use of seed-feeding agents had been proposed, as this had the potential to control the plant's spread without reducing its benefits, thereby subverting the problem of conflicts of interest (Neser & Klein 1998). Because leucaena was not a high priority weed, opportunities to collect potential agents were limited to scouting trips for agents of more problematic plants (Olckers 2004). So far, *A. macrophthalmus* is the only candidate agent that has been considered for biocontrol in South Africa.

Between April 2000 and June 2009, there were some 13 releases of the beetle in and around KwaZulu-Natal, including several releases around Durban, Pietermaritzburg and Mtubatuba (Olckers 2011). All releases have resulted in establishment, with the exception of a release at Cedara near Pietermaritzburg, where the site was cleared (Olckers 2011).

Besides deliberate releases in South Africa, the beetle has accidently been introduced into several other countries through contaminated seeds. The beetle was accidentally introduced to Australia prior to 1996, and was first recorded in Cyprus in 2007 (Raghu *et al*. 2005, Vassiliou & Papadoulis 2007). The beetle has also been reported from West Africa, where it attacks the seeds of cultivated leucaena trees (Delobel & Johnson 1998, Effowe *et al*. 2010). It has also been accidentally introduced into a number of Asian countries, including China, Japan, Taiwan, Thailand, Vietnam, Myanmar, and India (Tuda *et al*. 2009). Although leucaena is invasive in these countries, the introduction of the beetle was not welcomed as there were fears surrounding its host specificity and its potential to attack seeds of economically important legumes (Tuda *et al*. 2009). Indeed, there have been reports of non-target feeding on a few related legumes in Asia (Tuda *et al*. 2009, 2013) although this had not been observed in South Africa.

1.3.3. Seed damage

Seed damage by bruchine beetles (commonly referred to as bruchid beetles or bruchids in the literature) is notoriously variable, and often varies seasonally and spatially. A study by Raghu *et al*. (2005) in Queensland (Australia) looked at the seed damage caused by *A. macrophthalmus* in relation to pod retention times on the trees. Seed damage increased with pod retention times and ranged from 11% after one month to 54% after four months. However, the high seed production by the trees resulted in "predator satiation", and beetle numbers were not high enough to counteract this. Once the pods dehisced and the seeds were deposited on the soil surface, they then became less available to the beetles and escaped predation (Raghu *et al*. 2005). A study by Effowe *et al*. (2010) in West Africa (Togo) found a different trend. The highest infestation rates (72%) corresponded with periods of high pod production by leucaena trees, and infestation rates remained at this level for around four months before declining. Also, leucaena pods collected in Senegal (West Africa) had an infestation rate of 67% (Delobel & Johnson 1998). However, in Brazil, seed predation rates only reached 42% and beetle emergence coincided with leucaena's main fruiting period (Rodrigues *et al*. 2012). These levels of infestation suggest that the impact of *A. macrophthalmus* on leucaena populations in invaded countries has been variable and probably not extensive.

1.3.4. Parasitoids of *A. macrophthalmus*

Bruchid beetles in general are attacked by a range of parasitoids that target their eggs and endophagous immature stages (e.g. Kingsolver 2004). Various observations and studies have recorded both egg and larval parasitoids of *A. macrophthalmus* in countries where the beetle was introduced. In West Africa, larval parasitoids from the families Pteromalidae and Eurytomidae, as well as the egg parasitoids from the family Trichogrammatidae, were reared from collections of leucaena seeds (Delobel & Johnson 1998, Effowe *et al*. 2010). Shoba & Olckers (2010) reared two species of native chalcidoid wasps from leucaena seeds that were collected in the field in KwaZulu-Natal, South Africa. Native egg parasitoids (Trichogrammatidae) that normally utilize native bruchine species also contributed to high levels of egg mortality in KwaZulu-Natal (Ramanand & Olckers 2013). Parasitism levels

recorded by Raghu *et al*. (2005) in Australia were low, and were thought to have a negligible effect on beetle numbers. These instances of parasitism are presumably the result of parasitoids of native bruchine beetles incorporating *A. macrophthalmus* into their host range.

1.4. Pilot study in South Africa

A pilot study was conducted in KwaZulu-Natal, South Africa in 2010 to determine the levels of seed damage, as well as larval parasitism, in the field (Sharratt & Olckers 2012). Three sites along the KwaZulu-Natal coast (Fig. 1.2.), where beetles had previously been released on populations of leucaena (Olckers 2011) were sampled. The study was conducted from April to October of 2010, during the austral autumn/winter, when it was presumed that seed availability, and therefore seed damage, would be at its lowest.

Seed damage caused by the beetles during this time was erratic, and varied both spatially and temporally, ranging from 2-46% at Amanzimtoti, 10-49% at Durban and 2-62% at Verulam over the seven months (Sharratt & Olckers 2012). Overall, seed damage was low and averaged around 28% across all sites, over the course of the study, with damage levels seldom exceeding 50%. This was well below the 95% level of seed damage that is generally considered necessary to effectively control plant populations (Hoffmann & Moran 1998, Kriticos *et al*. 1999).

Five species of hymenopteran larval parasitoids, three of which were Pteromalidae, were recorded in the pilot study along with one species each of Eupelmidae and Eurytomidae (Sharratt & Olckers 2012). One of the species of Pteromalidae accounted for more than 50% of the larval parasitism. There was a moderately strong positive relationship between wasp numbers and beetle numbers, suggesting that parasitism was not incidental and that the beetles are actively being targeted by the wasps. Despite this, larval parasitism levels remained fairly low, averaging 7-9% at the three study sites (Sharratt & Olckers 2012), but were on average higher than recorded elsewhere on other bruchine biocontrol agents (Coetzer & Hoffmann 1997, Raghu *et al*. 2005, Zachariades *et al*. 2011) and may thus be having a negative effect on beetle populations.

Overall, the pilot study suggested that the beetles were ineffectual control agents, but highlighted the need for a longer study period that covered all four seasons, as beetle numbers were presumed to be their lowest during the winter months. Also missing from the pilot study was a consideration of the phenology of the plant, especially seed production, which could better explain seed damage levels caused by the beetles.

1.5. Aims of this study

Following the results of the pilot study, the aims of this study were primarily to determine the overall levels of seed damage suffered by leucaena populations, and how these levels of damage fluctuated over the year and between the four selected sites. To determine the effects of plant phenology on beetle numbers, flower and pod production by leucaena populations, was monitored monthly in relation to the levels of seed damage (Chapter 2). This was carried out to determine whether seed damage is driven by seed availability, and whether this relationship was density dependent (i.e. higher damage with higher seed availability) or inversely density dependent (i.e. lower damage with higher seed availability). Soil samples were also collected to determine the availability of seeds on the soil surface, as well as the proportion of these seeds that were damaged by the beetles (Chapter 2). To provide a more comprehensive record of larval parasitism of *A. macrophthalmus*, this study also recorded the extent of larval parasitism in the field and how this varied between sites and over seasons, as well as the relationship between parasitoid numbers and beetle numbers (Chapter 3). As native *Acacia* trees, which are relatively closely related to *L. leucocephala*, often grow in the vicinity of leucaena populations, they are thought to be the source of the parasitoids. In particular, parasitoids associated with native Bruchinae that infest the seeds of *Acacia* species are presumably pre-adapted to exploit *A. macrophthalmus*. Pods were collected from these trees to determine not only the extent to which the parasitoids of native bruchines overlap with those of *A. macrophthalmus*, but also whether *A. macrophthalmus* has remained host specific and confined to its target (Chapter 3).

CHAPTER 2: Phenology of *Leucaena leucocephala* populations and seed damage by *Acanthoscelides macrophthalmus*

2.1 Introduction

There are several characteristics of *L. leucocephala* that are shared with other invasive plants including continual (i.e. year round) seed production, a large seed bank and a high seed output (CABI 2014). The seeds produced by leucaena are dispersed by animals, including cattle, birds and rodents as well as humans (Delobel & Johnson 1998, Walton 2003). High seed producers spread quickly and can double their area of invasion within a year (Wilson & Flanagan 1991).

While not known as a major weed in South Africa, *L. leucocephala* has the potential to become a problematic invader because of its reproductive features (Neser & Klein 1998, Olckers 2011). It has thus been identified as an "emerging" weed with the potential to increase its invasiveness (Zimmermann & Neser 1999, Olckers 2004). Many of its seeds are released into the soil seed bank, which, as with other invasive legumes, is capable of containing tens of thousands of seeds that can remain in the soil for several years before germinating (Dennill *et al*. 1999, Hughes 2006). Because the trees produce seeds in such large numbers, mechanical and chemical control becomes difficult; especially once seeds are incorporated into the soil seed bank (Neser & Klein 1998). One study on another prolific seed producer, *Parkinsonia aculeata* L. (Fabaceae), found that, when assessing seed damage and germinability, mechanical control was counterproductive and destroyed existing bruchid populations, while encouraging germination of seeds in the seed bank through scarification (Cochard & Jackes 2005).

Biological control is therefore an attractive option for dealing with weeds that are high seed producers. *Acanthoscelides macrophthalmus* was introduced as a biocontrol agent of *L. leucocephala* specifically because it attacks only the seeds; the intention being to control the spread of the plant while still allowing farmers to cultivate it for fodder, as the vegetative structures of the plant are not damaged by the beetle (Neser & Klein 1998). Bruchid beetles consume at least 75% of the endosperm of an infested seed, destroying the cotyledon and therefore its ability to germinate, while not harming any other part of the plant (Wilson & Janzen 1972, Southgate 1978).

Assessing biocontrol agent densities (e.g. seed infestation levels) in relation to plant phenology (e.g. seed availability) is important for understanding the outcome of biological control operations. In particular, a lack of synchronization between the agents and the targeted plant tissues often leads to a lack of success (e.g. high numbers of seeds but low numbers of seed-feeders). Aspects of the phenology of other leguminous weeds have been examined in previous studies, such as within season seed availability in *Parkinsonia aculeata* in Australia (van Klinken 2005). Studies on *L. leucocephala* have looked at seed damage in Australia in relation to pod retention time, while others looked at infestation rates at different times of the year in Togo (Raghu *et al*. 2005, Effowe *et al*. 2010). However, while such studies focus on seed damage, they sometimes do not fully examine the reproductive capacity or monthly seed availability of the weed populations, which is important in understanding the dynamics between seed-feeding agents and the plant in the context of successful biological control (see above).

Seed-feeding bruchid beetles have been used in South Africa as biological control agents against leguminous weeds that are prolific seed producers, with *Algarobius prosopis* (Le Conte), *A. bottimeri* Kingsolver and *Neltumius arizonensis* (Schaeffer) released on *Prosopis* species, and *Sulcobruchus subsuturalis* (Pic) on *Caesalpinia decapetala* (Roth) Alston (Coetzer & Neser 1999, Impson *et al.* 1999). Neither of these programmes has met with major success and various reasons have been put forward to explain the failure of the bruchids to control their target plants. These include failure of the agents to establish, competition between agents, consumption of the seed pods by cattle and predation or parasitism of the immature stages (Impson *et al*. 1999, Byrne *et al.* 2011). Following the pilot study on *L. leucocephala* in 2010, it was decided that a more comprehensive evaluation of seed damage was required to provide a better understanding of why *A. macrophthalmus* has ostensibly been ineffective (Sharratt & Olckers 2012). The first aim of this study was thus to assess monthly seed damage in relation to the plant's phenology, notably monthly seed availability.

2.2 Methods

2.2.1 Study sites

The study sites were located in the coastal and midlands regions of KwaZulu-Natal (KZN), South Africa where healthy populations of leucaena occurred, and where the presence of *A. macrophthalmus* had previously been confirmed (Figure 1.2.). Three sites were located in the KZN coastal region which provided optimal climatic conditions for the plant and included: Verulam (29° 40' 46"S; 31° 02' 9"E); Durban (29° 49' 02"S; 30° 58' 59" E) and Amanzimtoti (30° 01' 53"S; 30° 53' 29" E). A fourth site was located at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29° 40' 05"S; 30° 24' 24"E), as it was at the edge of the expected range of *L. leucocephala* infestations. The sites were inspected monthly, from July 2011 to June 2012, during which assessments were made of the reproductive phenology of the plant populations and the levels of seed damage inflicted by *A. macrophthalmus* on canopy-held pods as well seeds that had dehisced from the pods onto the ground below the trees. A full year's set of data were obtained from these sites, with the exception of the Durban site where the trees were cut down in April 2012, allowing only 10 months of data collection.

2.2.2 Assessing the phenology of leucaena trees

The phenology of leucaena populations (notably seed availability) was assessed at each of the four study sites. Ten pod-bearing trees were randomly selected at each site at the beginning of the study. These trees were marked using spray paint so that the same trees and branches could be checked consistently during the monthly assessments. To measure the reproductive output of the plant populations, two branches on each tree were selected and marked, 0.75 m from the terminal end in order to include all reproductive material. At each sampling occasion, all reproductive structures, notably flowers and seed pods at various stages of development, were recorded on the marked part of the branches. Since this assessment was primarily aimed at determining seed availability, all floral material was combined into one category that included: the green, pre-flowering buds; fully-developed white flowers and; brown flowers in the post-flowering stage of development. The seed pods were recorded separately as: green pods; ripe, undehisced pods and; ripe, dehisced pods (Figure 1.1.). These numbers were then averaged between the two branches to

provide the average number of reproductive structures per branch for each tree, and thereby gain some insight into the phenological stage of the population.

2.2.3 Assessing damage to dehisced seeds on the soil surface

To assess the proportion of damaged seeds on the soil surface, 10 pod-bearing trees were selected every month, at each site. A 0.5 m x 0.5 m quadrat was placed on the ground at a distance of 0.5m from the base of the tree, under the tree canopy. Using a trowel, about 1 cm of the soil layer was removed from within the quadrat, placed in a Ziploc™ bag and returned to the insectary at the University of KwaZulu-Natal. The samples were then sieved and all seeds were removed and placed in Petri dishes to observe beetle emergence. The seeds were maintained in the Petri dishes for two months, during which time any beetles or wasps that emerged from the seeds were removed to prevent re-infestation. Thereafter, the number of seeds per sample, as well as the number of damaged seeds (i.e. with adult emergence holes), was recorded. During subsequent samples, care was taken to avoid re-sampling the same areas beneath the same trees.

2.2.4 Assessing damage to canopy-held seeds

Bruchid infestation levels were assessed monthly at each of the four study sites. Taking care to avoid the trees and branches that had been marked for the phenology study, 10 trees were selected at each site on each sampling occasion. On each of these 10 trees, 10 ripe, undehisced pods were removed and placed in Ziploc bags™ which were also returned to the insectary at the University of KwaZulu-Natal.

In the insectary, the pods from each tree were shelled and the seeds from all 10 pods were placed into Petri dishes. Emerging beetles and wasps were removed every two days to prevent re-infestation and thus skewing of the emergence data. Given the beetle's life cycle of around 35 days (see section 1.3.1), the Petri dishes were monitored for three months, after which it was presumed that very few (if any) more beetles were going to emerge. The monitoring of each Petri dish was terminated, following a period of 10 days during which no more beetles had emerged. Thereafter, the number of intact and damaged seeds, as well as the number of holes created by emerging beetles and parasitic wasps was recorded for each sample of 10 pods. Wasp emergence holes were easily distinguished from beetle emergence

holes due to their small size and the shape of the hole created by the wasps, which was more jagged. The number of holes created by the beetles and wasps was then equated to seed damage, as most seeds only contained one beetle or wasp. The parasitoid wasps that were removed were counted, separated according to different species (i.e. given accession numbers) and identified to family level using a key (Prinsloo 1980).

2.2.5 Statistical analysis

All analyses were conducted using IBM SPSS Statistics version 22.0. The number of beetle-damaged seeds as a percentage of the total number of available seeds was compared between months and sites, for both canopy-borne and soil-borne seeds. None of these data sets met the assumptions of normality and/or homogeneity of variances, despite attempts to transform them. As a result, generalized linear modelling was used to determine the influence of month and sites, and their interaction, on the beetle's damage to canopy-borne and soil-borne seeds. Since these models analysed binary data (counts of beetle-damaged seeds versus counts of available seeds), they incorporated a Binomial distribution and logit link function. Significance ($P < 0.05$) was assessed using Wald chi-square statistics. The relationship between the percentage of seeds damaged and the number of seeds available (Log_{10} of seeds per branch) per sampling occasion (i.e. pooled for the 10 trees) was determined using Spearman's rank-order correlation, since the assumptions of normality were not met.

2.3 Results

2.3.1. Reproductive phenology and seed availability

The reproductive phenology of leucaena was plotted to determine when the peak podding and flowering times occurred, and whether they were different between the sites and months (Figure 2.1). The number of seeds per pod was also plotted to determine whether this was consistent across sites and months, or if there was any variation (Figure 2.2). Seed availability was then plotted to determine the numbers of seeds that were available to *A. macrophthalmus* at each site over the course of a year (Figure 2.3).

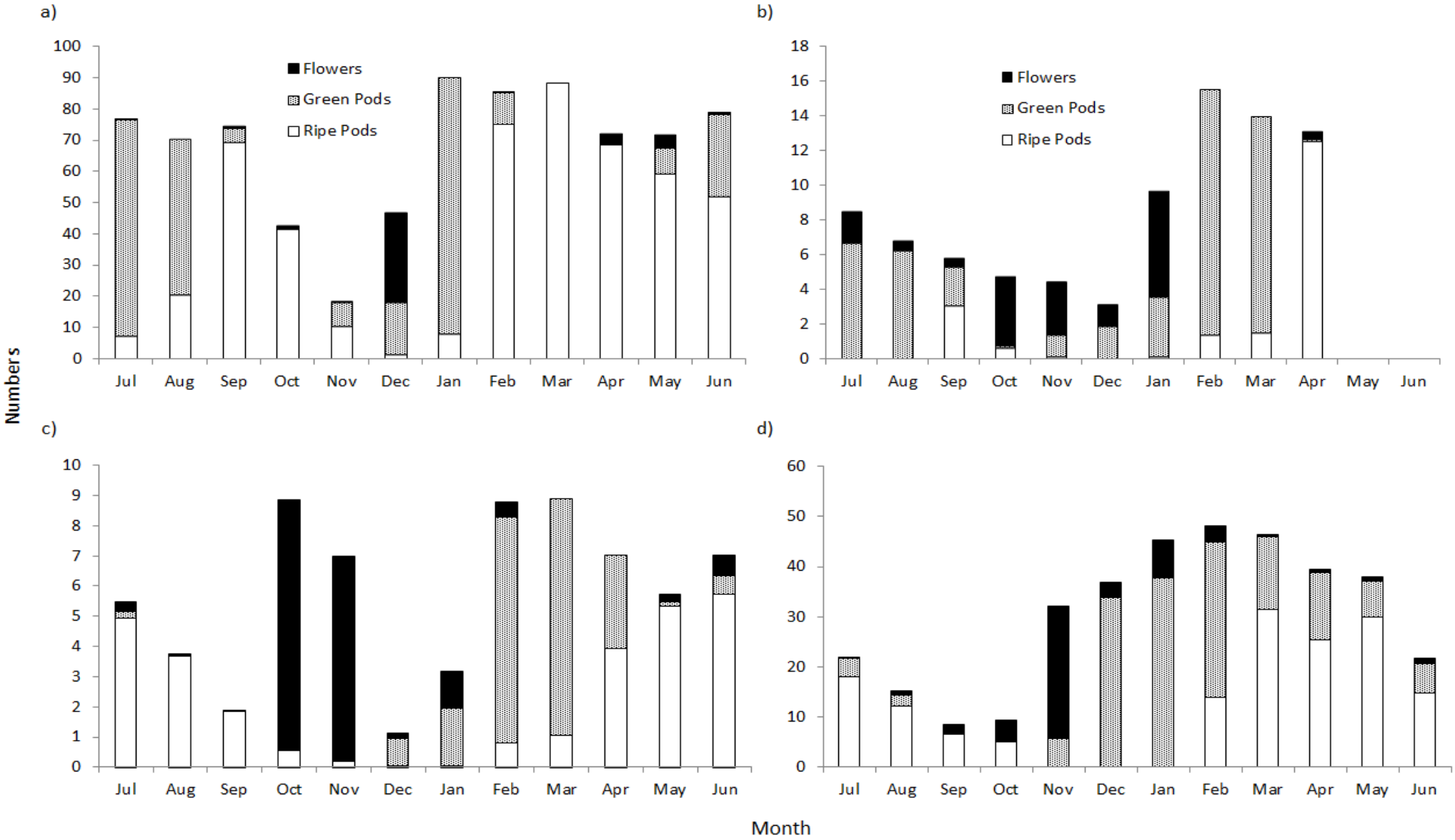

Figure 2.1 Mean numbers of reproductive structures per marked branch of *Leucaena leucocephala*, including all stages of flowers, green pods and ripe intact pods at the four KwaZulu-Natal sites, namely a) Verulam, b) Durban, c) Amanzimtoti and d) Pietermaritzburg, over the course of the 12-month study period.

The reproductive phenology of leucaena trees was assessed at all four sites over a period of 12 months, with the exception of the Durban site, which was only sampled for 10 months, giving a total of 46 sampling events (n = 460 trees) over 2011 and 2012. The Verulam site, with the oldest and largest trees, had the highest availability of ripe pods, ranging from a mean of 1 per branch in December to 88.5 in March (Figure 2.1). Trees at the Durban site, which were generally much smaller, produced no pods in July, August and December, and reached a peak in April with 12.6 ripe pods per branch (Figure 2.1). Trees at Amanzimtoti had the lowest mean number of ripe pods per branch, ranging from 0.1 in December and January to 5.8 in June (Figure 2.1). At Pietermaritzburg, pod numbers per branch ranged from 0.1 from November to January to 31.5 in March (Figure 2.1).

The patterns of pod availability were different at each site. At Verulam, there was an increase in the number of ripe pods from July to September, followed by a decrease until December and then an increase from January to March, and then a gradual decrease from April to July. There were no clear patterns at Durban, although minor peaks in pod production were observed in September and April. Trees at Amanzimtoti showed a steady decrease in pod numbers from July to December, followed by a steady increase from January to June. Trees at Pietermaritzburg also showed a steady decrease from July to November, followed by an increase from February to May. Despite these differences, all four sites experienced relatively lower numbers of ripe pods during the spring/summer months of November, December and January (Figure 2.1).

Green pods were present on the trees at all sites for 8-10 months of the year (Figure 2.1). Mean numbers of green pods per branch were usually high for two or three months of the year at each site, which preceded increases in the mean numbers of ripe pods. High numbers of green pods were in turn preceded by high numbers of flowers which were present on the trees at all sites for some 10-12 months of the year (Figure 2.1). Trees at Verulam displayed a peak in flower production in December, followed by a peak in green pod production in January. Flower production at the Durban site peaked in January, and was followed by peaks in green pod production in February and March. At Amanzimtoti, flower production peaked in October and November, but green pod production peaked much later in February and March. Flower production at the Pietermaritzburg site peaked in November, and was followed by peaks in green pod production in December and January.

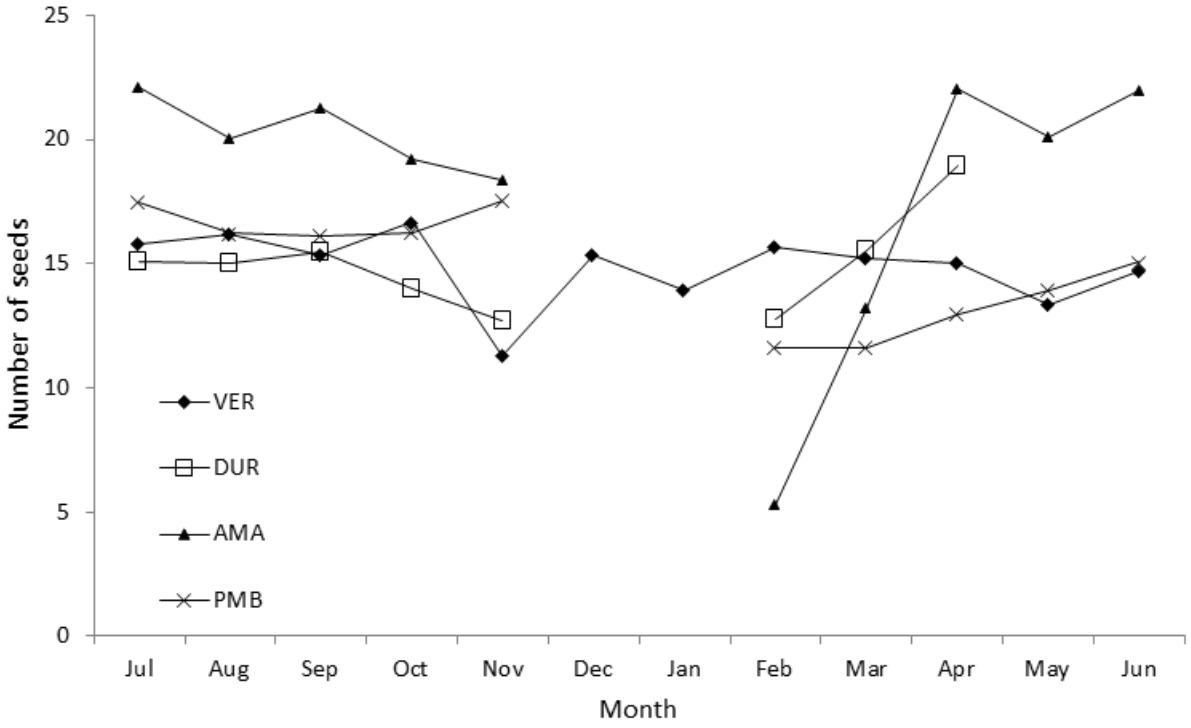

Figure 2.2 Mean numbers of *Leucaena leucocephala* seeds per pod at Verulam, Durban, Amanzimtoti and Pietermaritzburg over the 12-month study period.

In total, 3810 pods were collected from the four sites over the course of a year, yielding 59 849 seeds at an average (± S.E.) of 15.7 (± 4.2) seeds per pod. There were variations in the mean numbers of seeds per pod (Figure 2.2) between the different months and sites. The site with the highest number of seeds per pod was Amanzimtoti, with an overall mean (± S.E.) of 18.4 (± 5.3). Trees at Verulam (14.9 ± 1.4), Durban (14.9 ± 2.0) and Pietermaritzburg (14.9 ± 2.2) all had very similar mean numbers of seeds per pod. The trees at Amanzimtoti displayed the greatest variation in monthly seed numbers, with pods in September containing a mean of 21.2 seeds, and pods in February containing a mean of only 5.3 seeds. These variations in seed numbers per pod suggested that calculations of seed availability (see Figure 2.3 below) should not assume an average of 15.7 seeds per pods but need to incorporate these monthly/site variations (Figure 2.2).

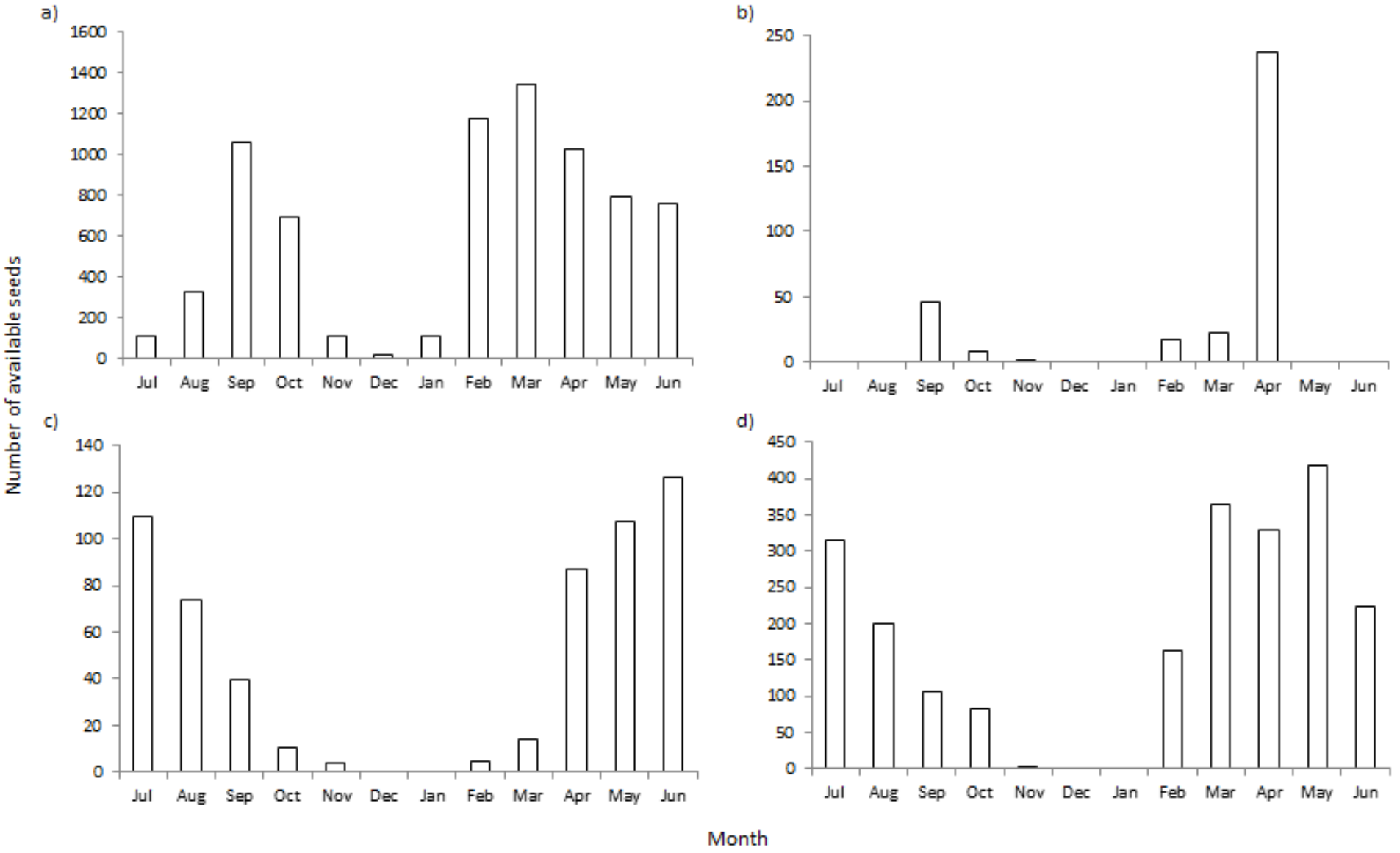

Figure 2.3 Mean numbers of *Leucaena leucocephala* seeds (per branch) that were available for *Acanthoscelides macrophthalmus* at a) Verulam, b) Durban, c) Amanzimtoti and d) Pietermaritzburg over the course of the 12-month study.

Seed availability at the four sites was thus determined by multiplying the mean number of seeds per pod (Figure 2.2) by the mean number of pods per branch for each month (Figure 2.1). There were considerable differences in seed availability between the four sites and over the 12 months within each site (Figure 2.3). The highest seed densities were observed at Verulam, where seed numbers peaked at 1345.2 seeds per branch in March. Seed densities were substantially lower at Pietermaritzburg, where numbers peaked at 462.4 seeds per branch in May. Seed densities were even lower at Durban (peaking at 237.7 seeds in April) and Amanzimtoti (peaking at 126.16 seeds in June), which was most likely a reflection of the younger trees in the populations. All four sites displayed very low seed availability from November to January, while no seeds were available during December and January at all sites except Verulam (Figure 2.3).

2.3.2 Canopy-held seed damage by *A. macrophthalmus*

Damage to canopy-held seeds of *L. leucocephala* by the larvae of A. *macrophthalmus* was examined to assess the impact of the beetle on the plant's reproductive capacity (Figure 2.4). Seed damage was also compared to the availability of leucaena seeds to determine whether seed availability influenced the levels of damage (Figure 2.5). The levels of seed damage were also compared between the pilot study in 2010 and this study (2011-2012), to determine whether damage fluctuates substantially between years, since three of the sites (Verulam, Durban and Amanzimtoti) were sampled during both studies (Figure 2.6).

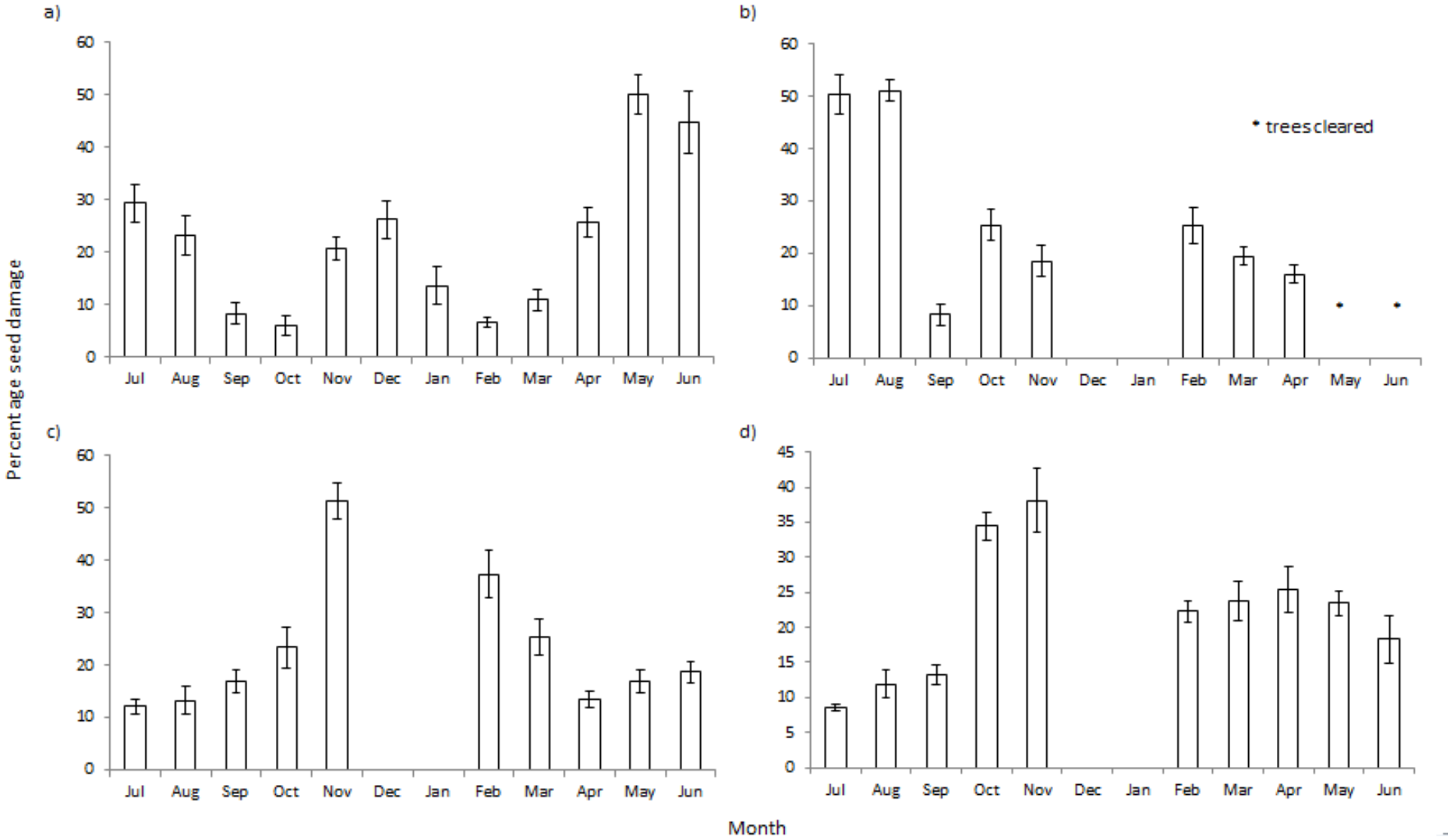

Figure 2.4 Mean (± S.E.) percentage seed damage for canopy-held seeds at a) Verulam, b) Durban, c) Amanzimtoti and d) Pietermaritzburg over the course of the 12-month study.

Of the 59 849 seeds collected over the 12 months at all four sites, some 10 068 (16.8%) were damaged by A. *macrophthalmus.* There were significant differences in seed damage (Figure 2.4) between months (Chi^2 = 161.374; df = 11, P < 0.0005) and sites (Chi^2 = 26.156; df = 3, P = 0.013) and the interaction between months and sites (Chi^2 = 949.375; df = 25, P < 0.0005) was also significant. No seeds were available for collection from Durban, Amanzimtoti or Pietermaritzburg during December and January.

The mean percentage seed damage was highly variable and erratic at all of the four sites (Figure 2.4). At Verulam, seed damage varied between 6.0 ± 1.8% in October to 50.1 ± 3.7% in May, with the highest levels of damage during May to July and the lowest during September, October and February. The Durban site's mean seed damage ranged from 8.4 ± 2.1% in September to 51.2 ± 2.0% in August, with the highest levels of damage during July and August and the lowest during September. Clearing of the trees at Durban during May precluded any further sampling. Seed damage at Amazimtoti ranged from 12.2 ± 1.4% in July to 51.2 ± 3.4% in November, with the highest levels of damage during November and February and the lowest during July, August and April. Seed damage at Pietermaritzburg ranged from 8.5 ± 0.5% in July to 38.1 ± 4.6% in November, with the highest levels of damage during October and November and the lowest during July and August. Overall, the mean percentage seed damage exceeded 50% on four occassions only (Figure 2.4), and seed damage was usually less than 30%.

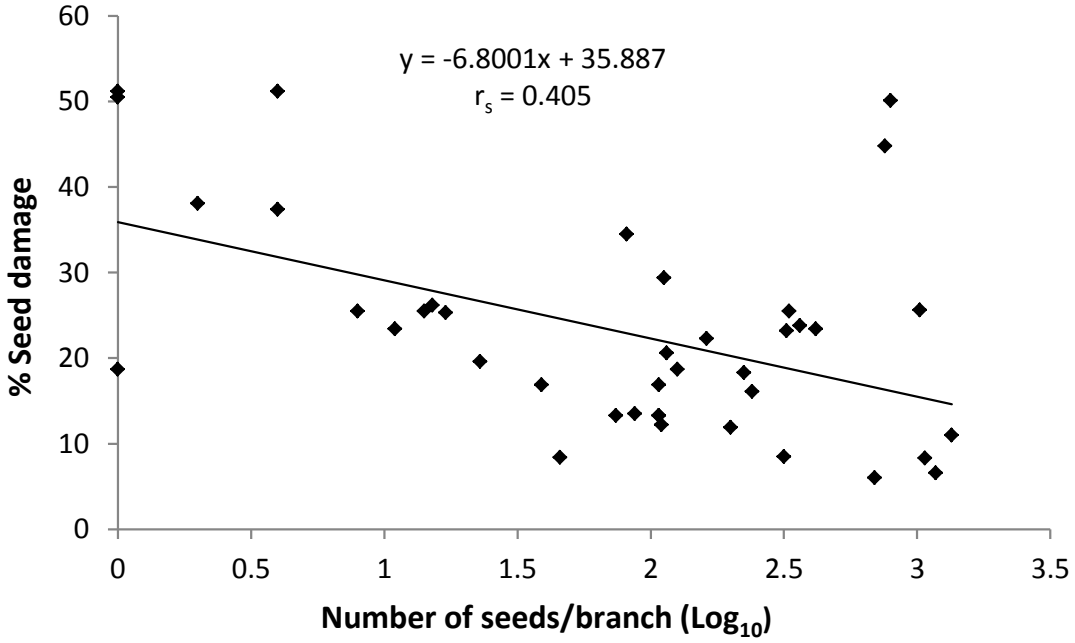

Figure 2.5 Relationship between the percentage of canopy-held seeds damaged by *Acanthoscelides macrophthalmus* and the number of available seeds per branch of *Leucaena leucocephala.* Data were pooled for the 10 trees sampled monthly at each site.

The mean monthly percentage seed damage at the four sites (Figure 2.4) was then plotted against the mean number of seeds that were available to the beetles during each month (Figure 2.3) to determine the relationship between seed damage and seed availability. There was a moderately strong, negative and significant correlation (r_s = 0.405; r^2 = 0.164; n = 40; P = 0.009) between percentage seed damage and seed availability, showing that the highest levels of seed damage occur when seeds are less abundant and vice versa (Figure 2.5).

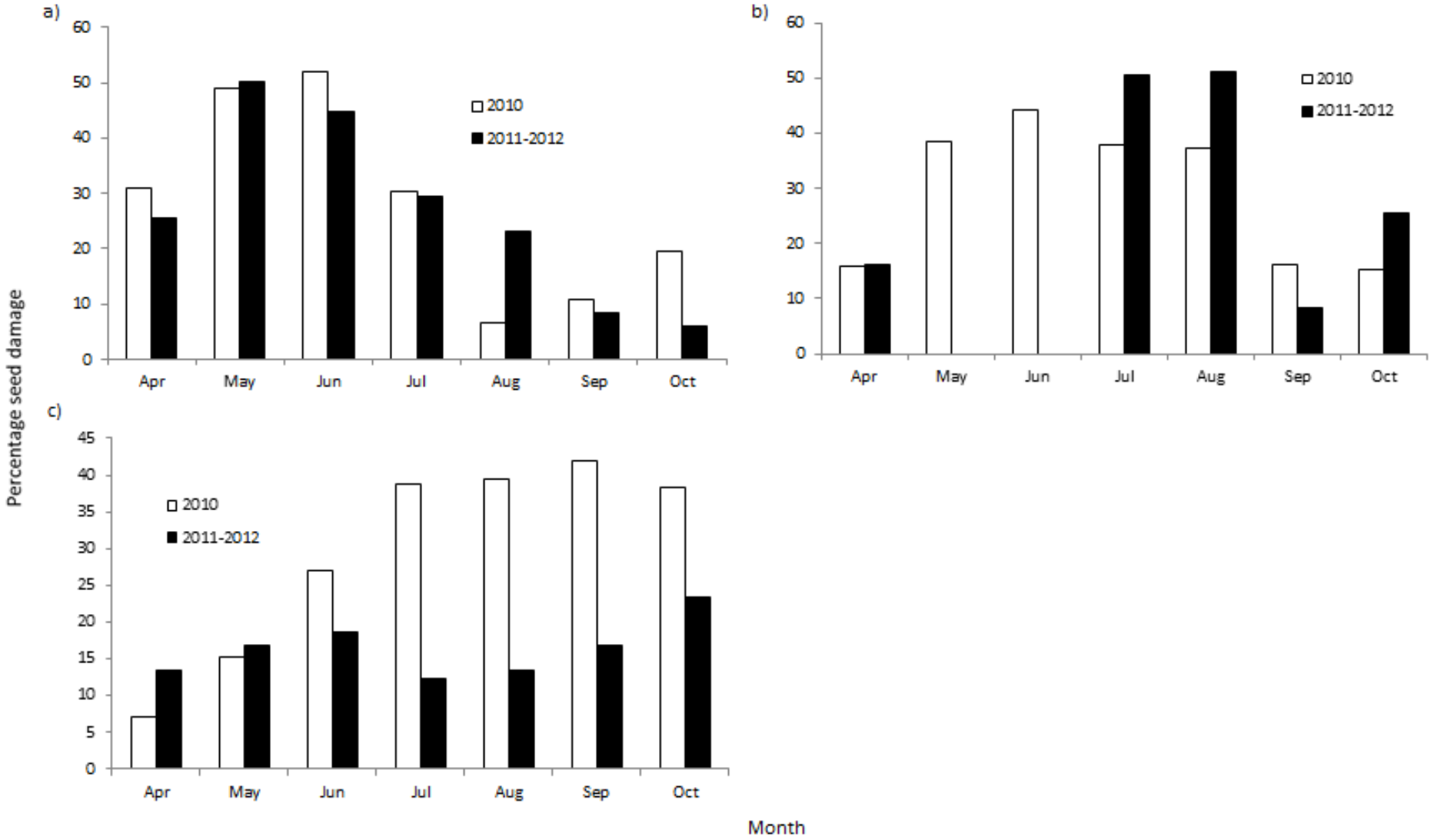

Figure 2.6 Mean percentage damage of canopy-held seeds of *Leucaena leucocephala* at a) Verulam, b) Durban and c) Amanzimtoti during the pilot study (2010) and the present study (2011-2012).

The present study was conducted over a 12-month period from the winter of 2011 to the winter of 2012. In the pilot study, however, only seven months of the year were sampled, and this was carried out from April to October 2010, incorporating autumn, winter and early spring. The monthly means of seed damage (April to October) were compared between these two studies to determine whether seed damage was consistent between years (Figure 2.6). At Verulam, the same population of *L. leucocephala* was sampled while at Durban and Amanzimtoti, different populations (albeit in close proximity to the original ones) were sampled due to the clearing of the trees at the original sites.

Over the same time frame (April to October) the mean levels of seed damage were generally lower in the present study than in the pilot study (Figure 2.6). In the pilot study, 210 trees were sampled, yielding 39 035 seeds, with 10 742 (27.5%) damaged by the beetle. In the present study, 190 trees were sampled over the same period (20 less due to the clearing of trees at the Durban site), yielding 33 213 seeds with 6 121 (18.4%) damaged by the beetle. At the Verulam site, the levels of seed damage were considerably higher during April to July in 2010 relative to the same months in 2011/12, reaching a peak of 51.9% in 2010 and only 23.4% in 2011/12 (Figure 2.6). The trend was different at the Durban site(s), where the levels of seed damage were higher during July, August and October in 2011/12 relative to the same months in 2010. At the Amanzimtoti site(s), the levels of seed damage were also considerably higher, but during June to October, in 2010 relative to the same months in 2011/12, reaching a peak of 42.0%, in 2010 and only 23.3% in 2011/12 (Figure 2.6). Seed damage was therefore inconsistent between the two studies.

2.3.3 Soil seed availability and damage by *A. macrophthalmus*

The mean numbers of seeds on the soil surface below the trees were compared between months and sites (Fig. 2.7) as were the percentages of these that were damaged by *A. macrophthalmus* (Figure 2.8). Beetle damage was also compared between canopy-held seeds and seeds on the soil surface (Figure 2.9).

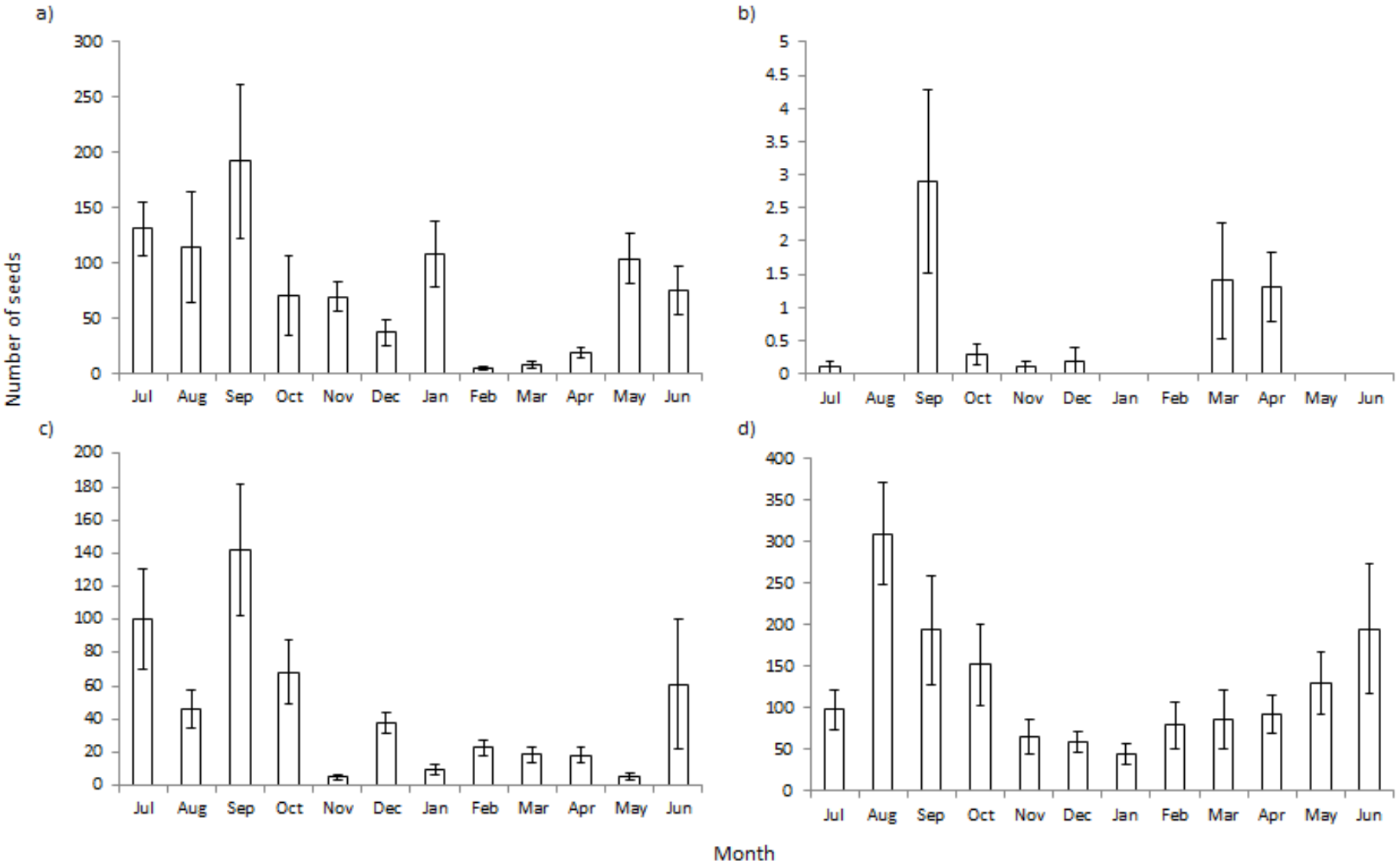

Figure 2.7 Mean (± S.E.) numbers of seeds of *Leucaena leucocephala* per quadrat (0.25m^2) on the soil surface at a) Verulam, b) Durban, c) Amanzimtoti and d) Pietermaritzburg over the course of the 12-month study.

A total of 29 773 seeds were collected from 480 quadrats (0.25m^2) placed on the soil surface at the four sites over the course of the 12-month study, with an overall average of 62 seeds per quadrat. There were substantial differences in soil seed densities (Figure 2.7) between months and sites.

At the Verulam site, mean soil seed numbers ranged from 4.9 ± 1.8 to 192.4 ± 69.8 seeds per quadrat in February and September, respectively. Soil seed densities were considerably lower at the Durban site where the trees were younger than at the other three sites. No seeds were recovered on five occasions, and the highest soil seed density (in September) was only 2.9 ± 1.4 seeds per quadrat. At Amanzimtoti, mean soil seed numbers ranged from 4.9 ± 1.9 to 141.8 ± 31.4 seeds per quadrat in November and September, respectively. At Pietermaritzburg, mean soil seed numbers ranged from 44.8 ± 12.8 to 309.4 ± 61.7 seeds per quadrat in January and August, respectively.

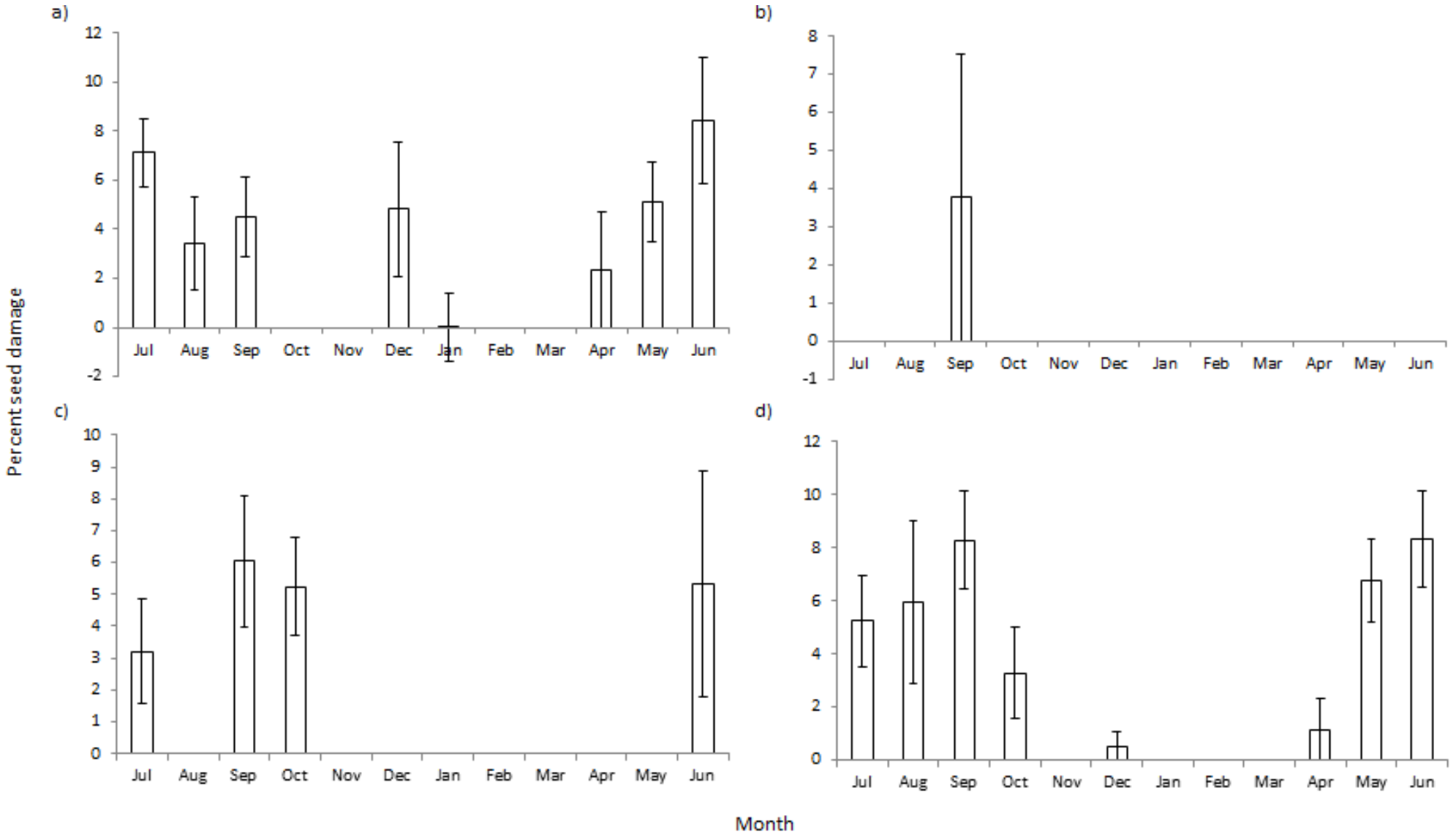

Figure 2.8 Mean (± S.E.) percentage seed damage (per 0.25m^2 quadrat) on the soil surface at a) Verulam, b) Durban, c) Amanzimtoti and d) Pietermaritzburg over the course of the 12-month study.

Of the 29 773 seeds that were recovered from the quadrats placed on the soil surface, only 418 (1.4%) were damaged by *A. macrophthalmus*. There were significant differences in soil seed damage (Figure 2.8) between months (Chi^2 = 12949.565; df = 11, P < 0.0005) and sites (Chi^2 = 2212.73; df = 3, P < 0.0005) and the interaction between months and sites (Chi^2 = 9738.434; df = 25, P < 0.0005) was also significant. In general, the levels of soil seed damage at the different sites were erratic, with no damage recorded in several months (Figure 2.8). At the Verulam site, soil seed damage varied from zero (recorded in four months) to a maximum of 8.4 ± 2.6% in June. At the Durban site, soil seed damage (<4%) was recorded in a single month (September), with zero damage in all other months. At the Amanzimtoti site, soil seed damage varied from zero (eight months) to a maximum of 6.0 ± 2.1% in September. At Pietermaritzburg, damage similarly varied from zero (four months) to a maximum of 8.3 ± 1.8% in June and September.

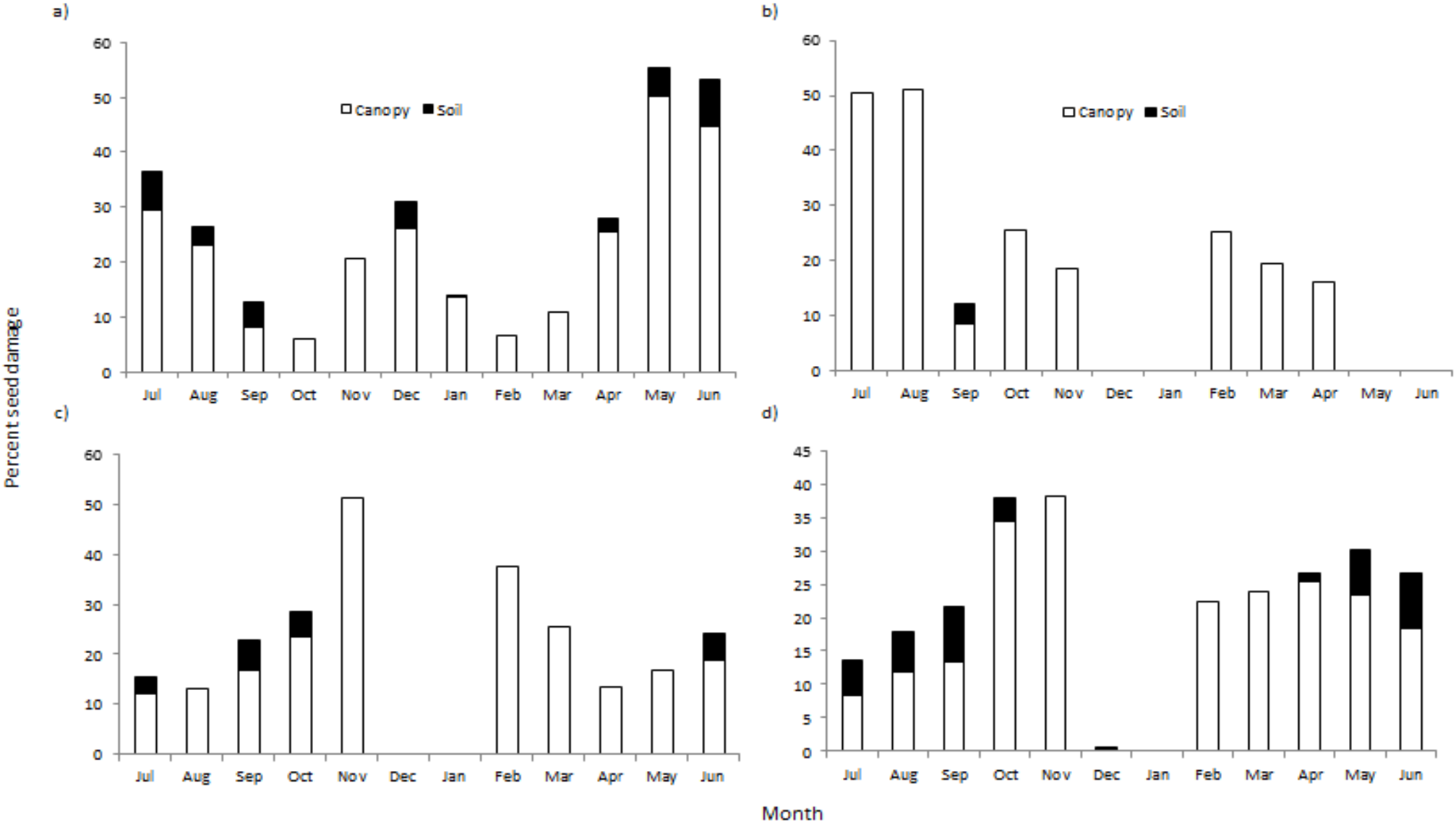

Figure 2.9 Mean percentage seed damage for canopy-held and soil-borne seeds at a) Verulam, b) Durban, c) Amanzimtoti and d) Pietermaritzburg over the course of the 12-month study.

When compared to canopy-held seeds, soil-borne seeds suffered considerably lower levels of damage by *A. macrophthalmus* (Figure 2.9). For example, the highest level for soil seed damage recorded during the entire study amounted to only 8.4 ± 2.6% compared with 51.2 ± 3.4% for canopy-held seeds. Soil seed damage was largely negligible and did not follow the same patterns as canopy seed damage. In particular, high levels of soil seed damage did not occur concurrently with high levels of canopy-held seed damage, or as delayed responses to changes in canopy seed damage (Figure 2.9).

2.4 Discussion

2.4.1 Phenology of leucaena

Larvae of *A. macrophthalmus* are not capable of developing in unripe seeds, and the adult females thus do not lay eggs on green pods that are suspended in the canopy (Neser & Klein 1998). Only ripe pods that are either on the trees or that are dislodged onto the ground are utilized by the beetle (Neser & Klein 1998; Egli & Olckers 2012). Depending on environmental conditions, the ripe pods dehisce readily (ripe but not dehisced pods shown in Figure 2.1), resulting in subsequent decreases in the numbers of intact ripe pods when green pods are present in low numbers. This tactic allows many seeds to escape predation by the beetles, since these do not readily attack loose seeds on the soil surface (see below).

When the pilot study was conducted, it was expected that ripe pods would be available throughout the year (Effowe *et al.* 2010), and that the trees' most prolific pod-producing months would be those during the austral summer, which were not sampled in the pilot study. However, this was not the case, as the months of November, December and January produced the lowest numbers of ripe pods, as the trees were mainly flowering or held high numbers of green pods. There were differences between the four sites in relation to the months of peak pod production, but the trees generally displayed high numbers of ripe pods from April to June (sometimes July). Effowe *et al.* (2010) found that the peak time for ripe pod production in West Africa (Togo), which has an equatorial climate, was from August to December, which was when pod numbers on local (i.e. KZN) leucaena were declining. Van

Klinken (2005) also found that although *Prosopis* seeds were available year round in Australia, there was a period of higher seed availability between October and January.

The number of seeds per ripe pod fluctuated throughout the course of the study, with differences between sites and months. A notable exception occurred during February at Amanzimtoti where substantially lower seed numbers were recorded due to many seeds in the pods being aborted (Figure 2.2). Consequently, calculations of seed availability took these differences in seed numbers into account when comparing the numbers of ripe pods per branch. Also, seed availability was based only on the numbers of ripe pods in the canopy, and excluded dehisced seeds on the soil surface (see below for explanation), unlike the study by van Klinken (2005), which took into account the non-dehiscent canopy- and ground-held pods when assessing seed availability. The low numbers of ripe leucaena pods during the summer resulted in relatively few to no seeds being available to *A. macrophthalmus* for around 90 days, which could have reduced its populations, as they have a generation time of 35-39 days (Effowe *et al.* 2010, Olckers 2011).

Seed availability is often believed to be the driving force behind variations in seed damage caused by bruchid beetles (Midgley & Bond 2001). However, other studies have argued that other factors are important (Ernst *et al.* 1989, Mucunguzi 1995, Raghu *et al.* 2005). One study found that despite the high numbers of *Acacia tortilis* (Forsk.) Hayne seeds being available to the bruchids over the course of their study, seed infestation still fluctuated greatly over the years (Ernst *et al.* 1989). The latter study also suggested that competition between the various seed predators caused these fluctuations. However, in the case of *L. leucocephala* in South Africa, a single seed predator is involved (no native bruchids were reared from any of the seeds during this study), thus ruling interspecific competition out as a possible factor.

2.4.2 Damage to canopy-held seeds

As previously reported (Sharratt & Olckers 2012), the levels of seed damage caused by *A. macrophthalmus* were well below the 95% level that is required to control leguminous weeds with high seed production (e.g. Hoffmann & Moran 1998, Kriticos *et al*. 1999). Seed predators thus need to inflict excessively high levels of damage in order to be effective, which some bruchid species are capable of achieving (van Klinken *et al*. 2009). One study

reported that bruchid damage inflicted on seeds of the native *Acacia tortilis* in Tanzania and Israel, reached 90-95% and 72-99%, respectively, resulting in only 1-3% germination of the seeds, although this may have been due to the effects of multiple seed feeders (Southgate 1978) and not a single insect species. However, such high levels of damage by *A. macrophthalmus* were never recorded during this study, let alone with any degree of consistency. As reported in the pilot study (Sharratt & Olckers 2012), seed damage levels were moderate over the entire period of this study and seldom exceeded 30% at any of the four sites. Indeed, the highest monthly seed damage levels were just over 50%.

Similar studies were conducted on the bruchid *Penthobruchus germaini* (Pic.) which was released for the control of *Parkinsonia aculeata* in Australia, where seed damage levels as high as 99% were recorded. However, as with *A. macrophthalmus*, *P. germaini* has had little success in controlling *P. aculeata*, as the levels of seed damage are inconsistent, and often fall well short of the required levels (Cochard & Jackes 2005). Low levels of bruchid seed damage were also reported by Coetzer & Hoffmann (1997), who recorded damage caused by *Neltumius arizonensis* and *Algarobius prosopis* on mesquite (*Prosopis* spp.) in South Africa. As with leucaena, a conflict of interests has arisen between farmers who use mesquite pods as livestock fodder and conservationists who consider the plant to be invasive. The latter study recorded the levels of seed damage on canopy-held and well as ground-held pods and reported that seed damage was seldom above 30% at any of the sites, approaching 60% on only two occasions. The suspicion that damage by bruchid biocontrol agents is often too low to control plant populations was reiterated by Radford *et al.* (2001) who studied the effects of the introduced bruchid *Bruchidius sahlbergi* Schilsky on *Acacia nilotica* subsp. *indica* (Benth.) Brenan in Australia. In this study, seed predation on canopy-held pods reached a maximum of only 32% over the three months of the study.

The slightly lower levels of seed damage in the present study, compared to that in the pilot study, highlights how variable seed damage caused by the beetles can be, despite sampling the same (or nearby) populations during the same seasons (Sharratt & Olckers 2012). Bruchid infestation levels are notoriously variable, with several studies documenting this trend. In the study of Ernst *et al.* (1989), infestation of *A. tortilis* seeds by bruchids (recorded on six trees at one site) varied between 37-82% in the first year, 10-24% the following year and 18-58% in the final year of the study, indicating high variability not just between

individual trees, but between subsequent years as well. The variability in seed damage reported in this and the pilot study is thus not surprising.

There was a moderate but negative relationship between seed damage and seed availability indicating an inversely density-dependent relationship. Higher numbers of available seeds result in a lower percentage of the seeds being damaged, thus thwarting biological control efforts. Trends like this are attributed to the agent's inability to reproduce fast enough during the target plant's peak fruiting periods (Cochard & Jackes 2005). As a result, seeds are able to escape attack due to "predator satiation", which results from the plants producing seeds in excess of predator population numbers, over a short period of time (Wilson & Janzen 1972, Raghu *et al.* 2005, Atlan *et al.* 2010). Higher levels of seed damage by *A. macrophthalmus* are thus not related to high seed production, but rather low seed production, as was also the case with this beetle in Australia (Raghu *et al.* 2005).

2.4.3 Soil seed damage

The number of seeds on the soil surface, while very low at the Durban site, were often much higher at the other sites, often exceeding 100 seeds per 0.25m^2 quadrat and reaching over 300 seeds during August at the Pietermaritzburg site. Although capable of locating dehisced seeds on the soil surface, *A. macrophthalmus* does not appear to utilize these to any extent (Neser & Klein 1998; Egli & Olckers 2012), limiting the number of available seeds and making it difficult for the beetle to build up adequate numbers during the periods when ripe pods are scarce or unavailable. *Mimosestes ulkei* (Horn), a bruchid released to control *Parkinsonia aculeata* in Australia, depends solely on canopy-held pods, while another bruchid, *Penthobruchus germaini*, utilizes both soil-borne and canopy-held pods (Cochard & Jackes 2005). Although these were non-dehiscent pods, retaining their seeds while on the soil surface, they were unutilized by *M. ulkei*, which is thought to be why populations of *P. germaini* persisted and those of *M. ulkei* did not (Cochard & Jackes 2005). Pods/seeds on the ground are often inaccessible to bruchid beetles by being buried in various substrates (e.g. soil, cattle dung) (Cochard & Jackes 2005). In a study of bruchid (*B. sahlbergi*) predation on *Acacia nilotica* seeds in Australia, less than 4% of the seeds collected from the soil were damaged (Radford *et al.* 2001).

The proportion of undamaged seeds on the soil surface effectively represents those that have escaped predation by *A. macrophthalmus*, and seeds that are dehisced quickly are thus an advantage to the plant population. Raghu *et al.* (2005) found that the number of *L. leucocephala* seeds that were available in the soil seed bank almost doubled over the 4 months of their study, while Cochard & Jakes (2005) found that 90% of *P. aculeata* pods had dropped onto the ground after two months. Raghu *et al.* (2005) also found no relationship between the number of available *L. leucocephala* seeds in the soil and the number of damaged seeds in the soil, which was consistent with the results of this study. The present study also found no relationship between the percentage of damaged seeds in the canopy and the percentage of damaged seeds on the soil surface. The very low number of damaged seeds on the soil surface indicates that a large portion of the seeds are escaping predation, possibly due to the rapid dehiscence of the pods and the relatively low numbers of canopy-held seeds that were damaged. Indeed, the damaged soil-borne seeds may have been a result of canopy-damaged seeds dehiscing and not undamaged seeds being located by the beetles on the ground. However, it is also possible that low recoveries of damaged seeds are the result of seeds degrading after being damaged by the beetles (Radford *et al.* 2001).

2.4.4 Conclusions

The results of both the present study and the pilot study revealed the erratic nature of *A. macrophthalmus* in achieving inconsistent, but generally low levels of seed damage (<30% of available seeds), despite an abundance of seeds during most months of the year. While competition with other seed-feeding agents has been highlighted as a reason for low levels of seed damage by some agents on invasive weeds (e.g. Impson & Hoffmann 1998), this is certainly not the case with this beetle as no other insect species have been reared from any of the field-collected *L. leucocephala* seeds (see Chapter 3).

There are a several possible reasons for the beetle's ineffectiveness in achieving high levels of seed damage. The beetle's inability to utilize unripe seeds or fully exploit dehisced seeds on the soil surface limits its potential as a biocontrol agent. In addition, it seems to be unable to respond to the plant's podding cycles in a density dependent manner and take advantage of increased seed availability. Other reasons include the influence of recruited

natural enemies, notably predators and parasitoids of the beetle's immature stages (see Chapter 3).

The inversely density-dependent relationship between seed damage by *A. macrophthalmus* and seed availability in the *L. leucocephala* canopy, coupled with the rapid dehiscence of pods, allows the majority of seeds to evade the beetle and limit its effectiveness. Although seed feeders, particularly bruchid beetles, are easy to implement in weed biological control operations, the levels of damage that they inflict are often not sufficient to control the weed populations on their own (e.g. van Klinken *et al.* 2009). However, despite *A. macrophthalmus* being incapable of regulating the numbers of leucaena plants (i.e. seedling recruitment), it may be capable of reducing the plant's rate of spread (van Klinken *et al*. 2009). Reducing a weed's rate of invasion can be achieved with much lower levels of seed damage, but depends on seed dispersal factors (e.g. how far the plant disperses its seeds) (van Klinken *et al*. 2009).

CHAPTER 3: Recruitment of native parasitoids and non-target effects of *A. macrophthalmus*

3.1 Introduction

Bruchid populations, whether they be native or introduced, can be influenced by a number of factors, including environmental conditions, their ability to track seed resources, egg mortality, larval competition within the seed and larval mortality through parasitism (van Klinken & Flack 2008). Hymenopteran parasitoids attack the various immature stages of bruchid beetles, including the eggs, larvae and pupae, and form the bulk of the beetles' natural enemies (Kingsolver 2004). The diversity of native bruchid beetles that are associated with native leguminous plants (e.g. *Acacia* species) (Van Tonder 1985; Impson *et al*. 1999) suggests that introduced bruchid species are likely to be susceptible to parasitism. Because of the wide host range of bruchid parasitoids, the recruitment by introduced bruchids of native wasps that normally parasitize native bruchid beetles is almost inevitable (Impson *et al.* 1999). Hymenopteran parasitoids are generally capable of attacking a number of host species, although their development and rates of parasitism often vary with the host (Ouantinam *et al.* 2006). However, as a general rule, there are mostly lower levels of parasitism on introduced hosts, as native parasitoids tend to prefer their native hosts to exotic ones (Torchin *et al.* 2003).

Parasitism can be affected by the abundance and health of the hosts that are available (Holling 1959). The ability of the parasitoids to regulate host populations will be a result of not only their interactions with the host population, but also their ability to maintain their own populations (Holling 1959). The rates of parasitism will thus be a result of a combination of these two factors (Holling 1959). Other factors affecting parasitoid populations include climatic conditions such as temperature and humidity, competition between parasitoids and hyperparasitism (van Alebeek *et al.* 1993, Oueadraogo *et al.* 1996, Ndoutoume *et al.* 2000).

Shoba & Olckers (2010) conducted a preliminary study on the native parasitoids affecting *A. macrophthalmus* in South Africa. Their study, which involved the exposure of beetle-infested seeds in the field, recovered only two species of parasitoids (both Pteromalidae), and at only one of the three sites where the seeds were exposed. Despite suggestions of low rates of parasitism, about half of the beetle-infested seeds that were exposed at this site

produced parasitoids. It was also noted that beetle emergence was slightly higher at the sites where there were no parasitoids present.

During the more intensive pilot study (Sharratt & Olckers 2012), five species of parasitoids were recovered with indications of a density-dependent relationship with the numbers of *A. macrophthalmus*. These observations suggested that parasitism could lead to a decrease in bruchid population numbers, which would in turn lead to a decrease in the agent's ability to control leucaena populations (Sharratt & Olckers 2012). The surveys of seed damage undertaken during this study (Chapter 2) were also used to verify the rates of parasitism, particularly since longer monitoring (and at additional sites) could result in the recovery of more parasitoid species, and determine the strength of the relationship between beetle larvae and their parasitoids.

Also, *A. macrophthalmus* was recently reported to attack the seeds of leguminous plants that were outside the genus *Leucaena* (but in the family Fabaceae), in the field in southern Asia (Tuda et al. 2009). Although no other examples involving bruchid biocontrol agents have been reported, such examples of host-range expansion in an agent's introduced country has created unease about the use of biological control. Considering the ease with which *A. macrophthalmus* spreads via contaminated seeds, the tendency of bruchids to become pests and claims that bruchids are capable of attacking non-target plants (Delobel and Johnson 1998, Amevoin et al. 2007, Tuda et al. 2009), an assessment of the beetle's host range in the field was deemed necessary. Since the genus *Acacia* (Fabaceae) is closely related to the genus *Leucaena*, native species of *Acacia* growing in close proximity to *L. leucocephala* stands were surveyed to determine whether their seeds were being utilized by *A. macrophthalmus*.

3.2 Methods

3.2.1 Collection and identification of parasitoids

Parasitoids that emerged from the *L. leucocephala* seeds that were collected over the 12 months at each of the four field sites (see Chapter 2 for details of sampling and recovery of beetles and parasitoids) were removed upon inspection and stored in vials in the freezer of

the insectary of the University of KwaZulu-Natal. Voucher specimens of the different species were prepared and these were then identified to family level using a key (Prinsloo 1980) and assigned an accession number.

3.2.2 Collection of native *Acacia* pods

During the collection of *L. leucocephala* pods in the field, pods of native *Acacia* trees that were in the immediate area were also collected. *Acacia* species were identified using Pooley's Trees of Eastern South Africa (Boon 2010) and included *Acacia nilotica* (L.) Willd. Ex Del., *Acacia sieberiana* DC. var. woodii (Burtt Davy) Keay and *Acacia karroo* Hayne and were sampled whenever pods were present on the trees. *Acacia nilotica* was present at the Verulam, Durban and Pietermaritzburg sites, *A. sieberiana* at the Pietermaritzburg site and *A. karroo* at the Amanzimtoti site. Since the intention of this survey was to determine the presence/absence of *A. macrophthalmus* and the source of the parasitoids that were associated with it, only one tree of each species was sampled on each occasion. During each collection, around 10 pods were collected from each tree (different trees sampled on each occasion), which were then placed in Ziploc™ bags and returned to the insectary. Overall, 44 pods were collected from *A. sieberiana* on four sampling occasions, 114 pods of *A. karroo* were collected on nine sampling occasions, and 332 pods of *A. nilotica* were collected on eight occasions. These pods were examined every two days, and any beetles or wasps that emerged were removed and placed in vials in the freezer for later identification. Voucher specimens of the beetles and wasps were prepared as before.

3.2.3 Statistical analysis

All analyses were conducted using IBM SPSS Statistics version 22.0. The number of parasitoid emergence holes as a percentage of the total number of infested seeds (determined by the presence of emergence holes of beetles and parasitoids) was compared between months and sites. Because the data were not normally distributed, generalized linear modelling was used to determine the influence of month and sites, and their interaction, on parasitism. Since the model analysed binary data (counts of parasitoids versus counts of beetle-infested seeds), it incorporated a Binomial distribution and logit link function. Significance ($P < 0.05$) was assessed using Wald chi-square statistics. The relationship between the total numbers of parasitoids and the total number of beetle-

infested seeds per sampling occasion (i.e. pooled for the 10 trees) was determined using Spearman's rank-order correlation, since (despite square root transformations) the assumptions of normality were not met.

3.3 Results

3.3.1 Parasitism of *A. macrophthalmus*

The percentage of emergence holes (i.e. that indicated the presence of an infested seed) that were caused by parasitoids was plotted to determine the extent and fluctuation of parasitism at the four sites throughout the year (Figure 3.1). The different parasitoid species (Figure 3.2) were then compared to determine the diversity and abundance of larval/pupal parasitoids (Table 3.1). Finally, the relationship between the number of parasitoid emergence holes and the total number of emergence holes (i.e. beetle holes and wasp holes, as the presence of a wasp indicates the presence of a beetle) was determined (Figure 3.3) to assess whether or not it was incidental.

Levels of parasitism reached a maximum of 39.2%, which was recorded in November at the Durban site, but otherwise did not often exceed 30% (Figure 3.1). Parasitism levels varied widely during the year at all sites, ranging from 4.4-27.7% at Verulam, 4.6-39.2% at Durban, 9.1-30.6% at Amanzimtoti and 5.7-31.1% at Pietermaritzburg (Figure 3.1). Consequently, there were significant differences in the percentages of beetle-infested seeds that were parasitized between months ($Chi^2 = 63.875$; df = 11, $P < 0.0005$) and sites ($Chi^2 = 10.794$; df = 3, $P = 0.013$) and the interaction between months and sites ($Chi^2 = 105.394$; df = 25, $P < 0.0005$) was also significant. The mean (± S.E.) percentage parasitism that was recorded over the whole study was 15.8 ± 0.1%. Parasitism was not recorded during December and January at the Durban, Amanzimtoti and Pietermaritzburg sites, since no pods were available during these months. The same applies to the last two months of sampling at the Durban site, since the trees were felled during May.

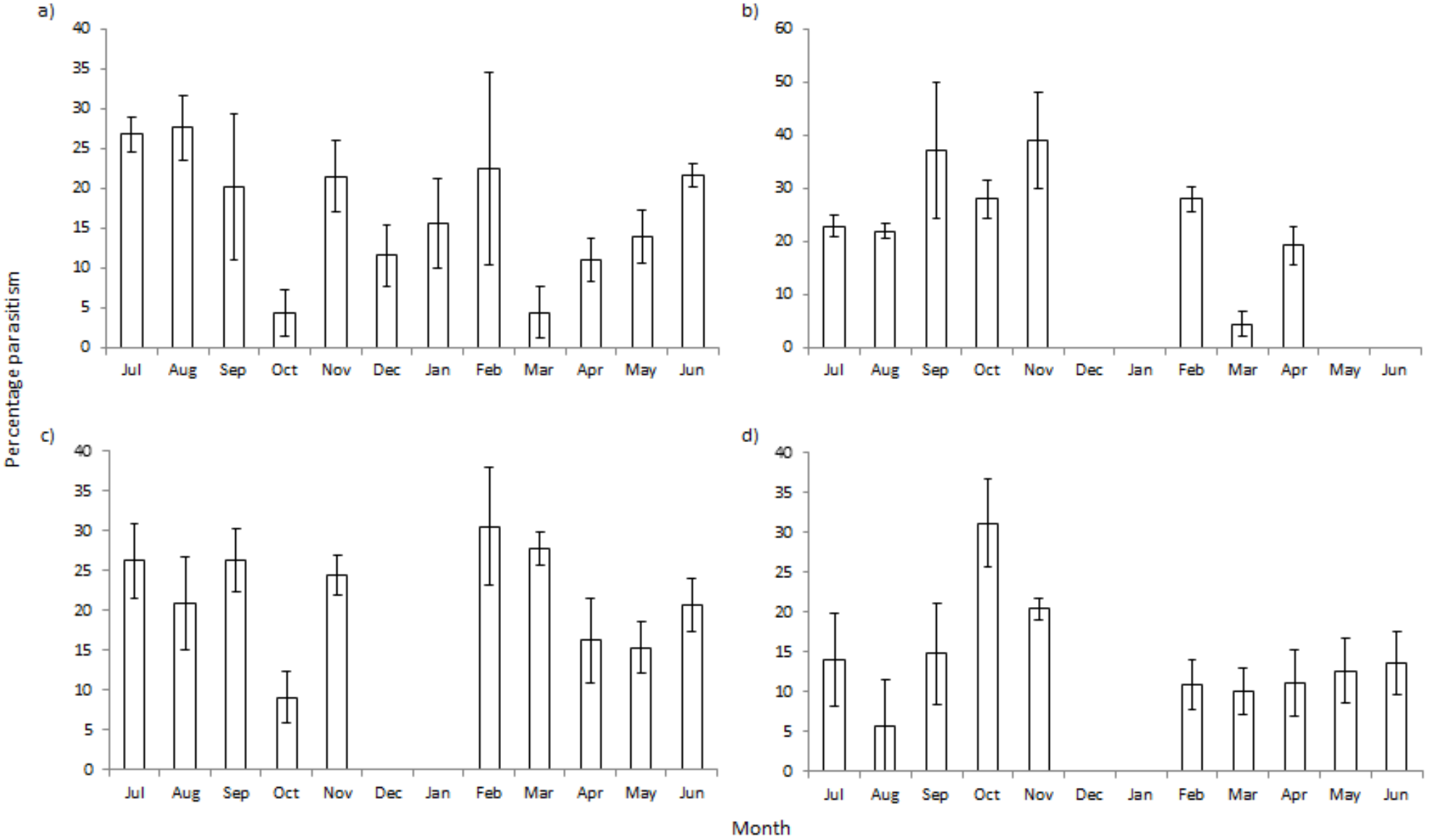

Figure 3.1 Mean (± S.E.) percentage parasitism of the larvae/pupae of *Acanthoscelides macrophthalmus* by native parasitoids at a) Verulam, b) Durban, c) Amanzimtoti and d) Pietermaritzburg over the course of the 12-month study.

Table 3.1 Total numbers of larval/pupal parasitoids of *Acanthoscelides macrophthalmus* reared from *Leucaena leucocephala* seeds (combined for the four sites) over the course of the 12-month study. See Fig. 3.2 for images of the parasitoids in relation to their accession numbers (AcTo 1-10).

	AcTo 1	AcTo 2	AcTo 3	AcTo 4	AcTo 5	AcTo 6	AcTo 7	AcTo 8	AcTo 9	AcTo 10	Species total*	% Species**
July	20	143	2	0	4	1	0	0	0	0	170	13.8
August	12	145	6	9	5	0	0	0	1	1	179	14.5
September	9	65	0	0	0	0	0	0	0	0	74	6.0
October	6	253	1	0	2	0	0	0	0	0	262	21.2
November	35	109	2	2	0	1	0	0	0	0	149	12.1
December	2	9	0	0	0	0	0	0	0	0	11	0.9
January	0	19	0	0	0	0	0	0	0	0	19	1.5
February	32	26	1	10	1	0	2	1	0	0	73	5.9
March	6	5	1	10	5	0	3	0	0	0	30	2.4
April	1	68	0	0	0	0	0	0	0	0	69	5.6
May	2	73	0	0	0	0	0	0	0	0	75	6.1
June	18	93	4	0	8	0	0	0	0	0	123	10.0
Monthly total	143	1008	17	31	25	2	5	1	1	1	1234	
% Monthly Total	11.6	81.7	1.4	2.5	2.0	0.2	0.4	0.1	0.1	0.1		

* Total number of individuals recovered for all parasitoid species during each month.

** Percentage contribution of each month's recoveries to the total number of parasitoids recovered.

Ten species of hymenopteran parasitoids (all in the superfamily Chalcidoidea; Figure 3.2) were recovered from beetle-infested seeds of *L. leucocephala* over the duration of the study (Table 3.1). In total, 1234 specimens were reared from the seeds over this time. The parasitoid families included Pteromalidae (AcTo 1, 2 and 3), Eupelmidae (AcTo 4 and 8), Eurytomidae (AcTo 5) and Eulopidae (AcTo 7), with three species arising from single specimens that were not identified to family level (AcTo 6, 9 and 10). The vast majority of the specimens collected (94.7%) belonged to the Pteromalidae, with one species (AcTo 2, Figure 3.2 b) constituting 81.7% of the specimens (Table 3.1). The dominant parasitoid was more than seven times more common than the next most abundant species (AcTo 1, Figure 3.2a) which comprised 11.6% of the specimens. A species of Eupelmidae (AcTo 4, Figure 3.2d), Eurytomidae (AcTo 5, Figure 3.2e&f) and Pteromelidae (AcTo 3, Figure 3.2c) which comprised 2.5%, 2% and 1.4% of the specimens, respectively, were the next most abundant parasitoids (Table 3.1). The remaining five species were recorded in very low numbers.

Parasitoids were recovered during all months of the year in which beetle-infested seeds were collected (Table 3.1). However, most of the specimens were recovered during winter and spring, as seen in the months of October (21.2%), August (14.5%), July (13.8%), November (12.1%) and June (10%). Recoveries were generally much lower during summer and autumn, with the lowest recoveries in December (0.9%).

Figure 3.2 Most commonly recovered larval/pupal parasitoids of *Acanthoscelides macrophthalmus* (all in the superfamily Chalcidoidea) comprising: a) AcTo 1 (Pteromalidae); b) AcTo 2 (Pteromalidae); c) AcTo 3 (Pteromalidae); d) AcTo 4 (Eupelmidae); e) AcTo 5 (Eurytomidae, female), f) AcTo 5 (Eurytomidae, male) and; g) AcTo 7 (Eulophidae).

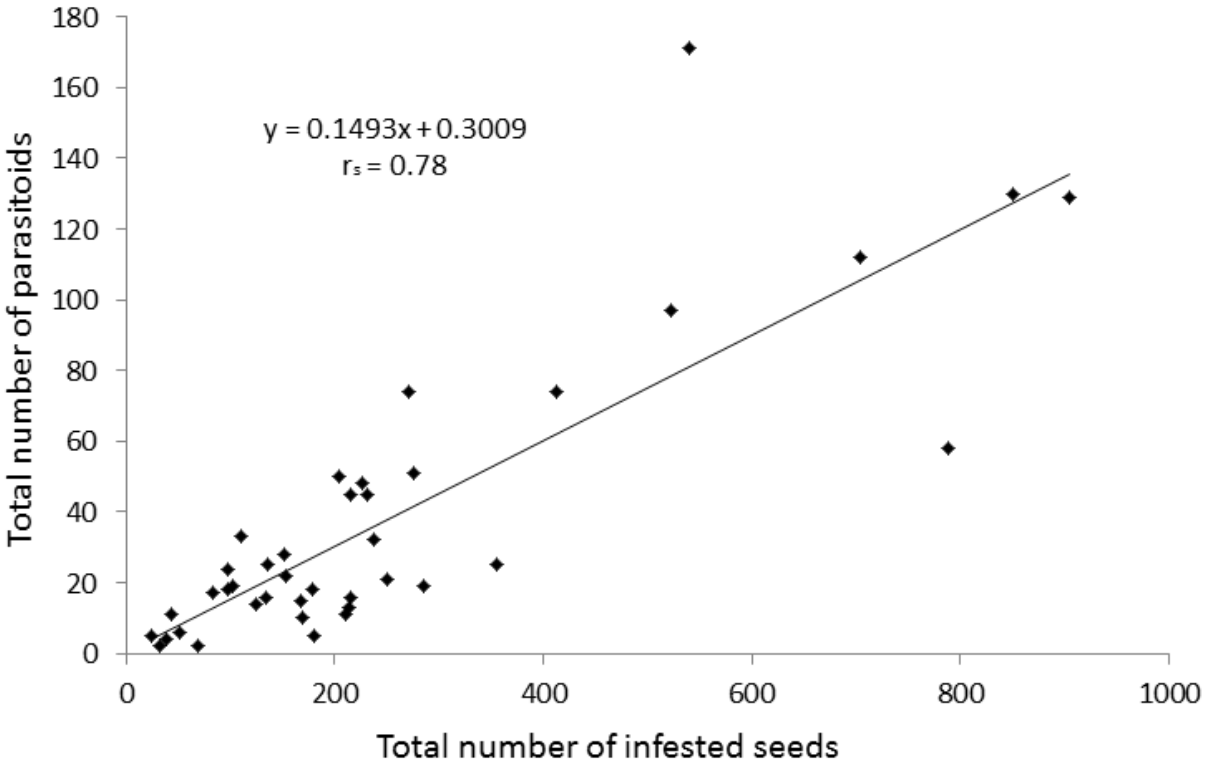

Figure 3.3 Relationship between the total number of parasitoids that emerged and the total number of beetle-infested seeds of *Leucaena leucocephala*. Data were pooled for the 10 trees sampled monthly at each site.

There was a strong, positive and significant correlation (r_s = 0.78; r^2 = 0. 61; n = 40; P < 0.0005) between larval/pupal parasitism and the availability of beetle-infested seeds.

3.3.2 Non-target effects of *A. macrophthalmus*

Over the duration of the study, some 490 pods of native *Acacia* species were collected to confirm the presence/absence of *A. macrophthalmus* in their seeds, but also to determine whether the parasitoids that were recovered from *L. leucocephala* seeds were recruited from those that attack native bruchids. Three species of *Acacia* were recorded in close proximity to the four *L. leucocephala* populations that were sampled monthly, namely *A. nilotica, A. sieberiana* and *A. karroo*. More pods were sampled from *A. nilotica* (332 pods) and *A. karroo* (114) than on *A. sieberiana* (44) due to greater pod availability during the monthly samples and the presence of the former species at three of the four study sites.

Table 3.2 Total numbers of individuals of larval/pupal parasitoids (AcTo = parasitoid accession numbers) and bruchid beetles that were reared from the pods of native species of *Acacia* that were in close proximity to the *Leucaena leucocephala* populations at the four sites (numbers combined for sites and months). The total numbers of pods sampled over the year are indicated.

	AcTo 1	AcTo 2	AcTo 4	AcTo 5	AcTo 7	AcTo 12	Native bruchids	*A. macrophthalmus*	Pods
A. sieberiana	0	0	0	0	3	22	27	0	44
A. nilotica	4	1	0	12	0	23	136	0	332
A. karroo	1	0	2	20	6	0	1	0	114
Total	5	1	2	32	9	45	164	0	490

None of the sampled pods showed any signs of infestation by *A. macrophthalmus* and not a single adult specimen was recovered from any of the native *Acacia* pods sampled during the course of the study (Table 3.2). At least three species of native bruchids (Table 3.2) were reared from these *Acacia* pods, mostly from *A. nilotica* (136 specimens) and *A. sieberiana* (27 specimens). Six species of hymenopteran parasitoids (also in the superfamily Chalcidoidea) were collected from pods of the three *Acacia* species, five of which (AcTo 1, 2, 4, 5 and 7; see Figure 3.2) were recovered from the seeds of *L. leucocephala*, with only one species (AcTo 12) not collected before. The pteromalid AcTo 2, which was the dominant species in *L. leucocephala* seeds (Table 3.1), was only collected once on *A. nilotica*, while the eurytomid AcTo 5, which was considerably less common in *L. leucocephala* seeds, was collected in higher numbers on both *A. nilotica* and *A. karroo* (Table 3.2). The dominant parasitoid species in the seeds of native *Acacia* species was the previously unrecorded species (AcTo 12).

3.4 Discussion

3.4.1 Larval parasitism

Populations of bruchid biocontrol agents, and consequently their ability to inflict seed damage, can be influenced by the recruitment of parasitoids that normally attack the immature stages of native bruchid species (Impson *et al.* 1999, van Klinken & Flack 2008). However, the regulation of insect populations occurs as a result of a density-dependent

factor, such as parasitism, which often results from parasitic wasps with wide host ranges which attack either their egg or larval stages (Holling 1959, van Klinken 2005). In particular, many species of parasitoid have been associated with bruchid biological control agents, with the more common families comprising Pteromalidae, Eulophidae and Eupelmidae (Moyal 1998, Ndoutoume *et al*. 2000, Briano *et al*. 2002, Shoba & Olckers 2010). In this study, 10 species of hymenopteran parasitoids emerged from *L. leucocephala* seeds, more than the two collected by Shoba & Olckers (2010) and the five collected during the pilot study (Sharratt & Olckers 2012). Pteromalidae comprised the bulk of the parasitoids associated with *A. macrophthalmus*, with the remaining families contributing little to parasitism. In particular, a single species of Pteromalidae comprised 81.7% of the recovered parasitoids. Dominance by one species is often the case with larval parasitoids; in the case of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), one of the five parasitoids was responsible for 91% of the parasitism (Moyal 1998).

Certain bruchid parasitoids perform better in the absence of interspecific competition, as observed with *Dinarmus basalis* Rond. (Hymenoptera: Pteromalidae), a larval parasitoid of *A. macrophthalmus* in West Africa (Gauthier *et al*. 1996). However, in situations with multiple species of parasitoids, the most competitive species, in this case *Eupelmus vuilleti* (Crawford) (Hymenoptera: Eupelmidae), was more dominant (Gauthier *et al*. 1996). In two bruchid-parasitoid systems, interspecific competition between *D. basalis* and *E. vuilleti* caused a decrease in the numbers of *D. basalis*, which, by itself, was the more effective of the two parasitoids at reducing bruchid numbers (Monge *et al*. 1995). In the present study, one species of Pteromalidae (Acto 2) appeared to more effective at exploiting the immature stages of *A. macrophthalmus*.

In the case of biological control agents, parasitoids are normally recruited from closely related native species, as a result of a broad host range, although their development on the new host is not always equivalent to that on the native ones (Ouantinam *et al*. 2006). In the present study, the four most abundant parasitoids that emerged from beetle-infested *L. leucocephala* seeds were recovered from the seeds of native *Acacia* trees, suggesting that native bruchids associated with these are the source of the parasitoids. The match between the parasitoids of *A. macrophthalmus* and those of native bruchids was not perfect, presumably because of limited sampling, with five species that were associated with *A.*

macrophthalmus not recovered from native *Acacia* seeds and one species that was associated with native acacias not recovered from *A. macrophthalmus*. This is not surprising since some species were recorded on *L. leucocephala* in very low numbers (sometimes only single specimens) and may be only incidental associates. Often, native parasitoids prefer native hosts to introduced ones (Torchin *et al*. 2003) but may perform equally well on exotic hosts. For example, *D. basalis* is usually associated with *Callosobruchus maculatus* (Fabricius), a common pest of cultivated Fabaceae, but displays similar rates of reproduction, egg laying, and parasitism on *A. macrophthalmus*, due to the beetles' similar life histories (Ouantinam *et al*. 2006, Effowe *et al*. 2010). Effective utilization of *A. macrophthalmus* as a host seems very likely to have a negative effect on the beetle's ability to control *L. leucocephala*.

Parasitoids were associated with *A. macrophthalmus* throughout the year, with rates of parasitism varying considerably between sites and months of the year. On average, around 15% of beetle-infested seeds yielded parasitoids and parasitism seldom exceeded 30%. However, this is fairly high in relation to parasitism that was recorded on other bruchids in other studies. Parasitism of bruchid larvae that attacked the seeds of *Acacia tortillis* only reached 6% and was insufficient to affect their populations (Ernst *et al*. 1989). Larval parasitism of two bruchids, *Algarobius prosopis* and *Neltumius arizonensis,* introduced for the biological control of *Prosopis* species in South Africa, was even lower, at <4 % (Coetzer & Hoffmann 1997). Larval and pupal parasitism rates in the maize stalk borer, *B. fusca*, reached a maximum of 9.1% and were similarly considered to be too low to influence the population dynamics of the moth (Moyal 1998).

The levels of parasitism recorded on *A. macrophthalmus* in this study were considerably higher than that reported for the beetle in Australia, where levels ranged between 0.4-1.1% over the study period (Raghu *et al*. 2005). In this study, there was a strong positive correlation between parasitism and the availability of beetle-infested seeds, confirming that this density-dependent relationship was not incidental and that the parasitoids are actively targeting the beetles (Sharratt & Olckers 2012). This may have been influenced by the proximity of the leucaena trees to native *Acacia* species. A similar relationship was reported between the parasitoids *D. basalis* and *E. vuilleti* and the bruchid *C. maculatus* (Monge & Huignard 1991). Once *D. basalis* was introduced to the system, the numbers of *C. maculatus*

decreased (Oueadraogo et al. 1996), and it is possible that the same pattern occurs with *A. macrophthalmus*.

3.4.2 Non-target effects

Host-range tests on *A. macrophthalmus* have been conducted a number of times, both before and after its release in South Africa (Neser & Klein 1998, Shoba & Olckers 2010). However, these tests were conducted in a laboratory, under controlled conditions, and no field assessments of the beetle's host range have previously been conducted. Non-target effects involving bruchids were first observed with *Bruchidius villosus* (Fabricius) (Coleoptera: Chrysomelidae), which was released for the control of *Cytisus scoparius* L. Link (Fabaceae) in New Zealand (Sheppard *et al*. 2006). Although these effects involved an exotic and not a native plant, this occurred despite host-range tests conducted in quarantine (Syrett & O'Donnell 1987).

Collections of pods of three species of *Acacia*, which belong to the same family as *L. leucocephala* and grew in close proximity to monitored infestations, revealed no signs of infestation by *A. macrophthalmus*, with only native bruchid species reared from them. This, along with the most recent host-specificity tests conducted on the beetle (Shoba & Olckers 2010), should put aside any fears, at least in the South African context, that the beetle will have undesired non-target effects. It is possible that the beetle that was inadvertently introduced into southern Asia and was linked with non-target effects (Tuda *et al*. 2009) represents a different biotype of *A. macrophthalmus*, with a different host range, to the material that was tested and released in South Africa, but genetic comparisons are needed to confirm this.

3.4.3 Conclusion

van Klinken & Flack (2008) suggested that multivoltine seed feeders, such as *A. macrophthalmus*, are less effective at tracking seed resources during the year, therefore causing less seed damage than univoltine seed feeders which are more synchronized with seed production cycles. This is presumably because the host plants of univoltine seed feeders have single podding cycles during the year, making synchronization essential and

therefore maximizing seed damage. However, in the case of *L. leucocephala* which sets pods throughout most of the year, a univoltine seed feeder would be considerably less effective than a multivoltine species, despite the shortcomings of the latter. In any event, an inability to track seed resources and variable seed availability was considered to be the most important factor affecting seed predator populations and levels of seed damage, with egg and larval mortality being less important (de Steven 1981, van Klinken & Flack 2008). The inversely density-dependent relationship between seed damage by *A. macrophthalmus* and *L. leucocephala* seed availability (Chapter 2) supports this contention. However, the density-dependent relationship between parasitoid numbers and the availability of beetle-infested seeds, suggests they have the ability to regulate *A. macrophthalmus* populations and are probably disrupting the beetle's field impact. There was no evidence of non-target effects by *A. macrophthalmus* in the field.

CHAPTER 4: General discussion and conclusions

4.1 Introduction

All of Africa, Asia, Europe, North and South America and Australia have native species of seed beetles (largely referred to as bruchids in the literature), as well as ones that were accidentally introduced via agricultural crops; these mostly attack the seeds of plants in the family Fabaceae (Southgate 1978, Kingsolver 2004). Bruchid beetles have been released as early as 1954 for the biological control of weedy plants (Krauss 1962; see Appendix 1). In theory, there are several reasons to suggest that bruchid beetles should be highly effective as biocontrol agents for limiting the reproductive output of plants that produce high numbers of seeds. Bruchid beetles are robust insects with rapid life cycles, high rates of dispersal and high fecundity, while damage to the seeds' cotyledons and endosperm, caused by the bruchid larvae during their development, inhibits the seeds' ability to germinate (Miller 1994).

The initial assessment of *A. macrophthalmus* in South Africa expressed hope that the beetle could curb the invasive potential of *L. leucocephala* by limiting its spread, while not undermining any of its useful attributes (Neser & Klein 1998). Although Olckers (2004) reported that five years after release, the beetle had not shown any signs of success against the plant, population level impacts of seed feeders are not immediately visible and it is often difficult to establish how effective they are as biological control agents (Impson *et al*. 2001).

4.2 Seed beetles as biological control agents

4.2.1 Efficacy in weed control

Bruchid beetles have been deployed as seed-feeding biocontrol agents against several invasive weed species worldwide (Appendix 1). While these have largely targeted weeds in the family Fabaceae, plants in the families Anacardiaceae, Lamiaceae, Malvaceae and Mimosaceae have also been targeted. Some 15 bruchid species have been released against nine weed species in 11 countries around the world, mostly in Australia (11 species) and

South Africa (5 species) (Appendix 1). Of the five species released in South Africa, three were released for various *Prosopis* species (mesquite) and one each for *Caesalpinia decapetala* (Roth) Alston and *Leucaena leucocephala*. Despite high hopes for bruchid agents on their respective targets, none of the 15 species released around the world have caused more than moderate levels of damage to their targets, with either negligible or unknown degrees of control reported (Appendix 1).

Two bruchid beetles were introduced into South Africa to target the ripe pods of mesquite, one in 1987 and another in 1990 (Coetzer & Hoffmann 1997). Only one of these, *Algarobius prosopis* (Le Conte), managed to establish, while post-release numbers of *Algarobius bottimeri* Kingsolver declined until it was no longer found in the field (Impson *et al*. 1999). Mesquite, which produces high numbers of seeds, is widely used as fodder in livestock production. The bruchid larvae develop inside the seeds which are often consumed by livestock, and as a result, many beetles are lost to livestock grazing (Coetzer & Hoffmann 1997). Another bruchid, *Neltumius arizonensis* (Schaeffer), was then introduced to combat this problem, as it was presumed to attack immature (green) pods, thereby giving the larvae time to develop and destroy the seeds before the cattle eat the ripe pods. However, since its release in 1993 it was discovered to actually attack the ripe pods and has thus had little success; coupled with its low abundance, which was aggravated by egg parasitoids (Coetzer & Hoffmann 1997). *Algarobius prosopis* damaged reasonable numbers of seeds, but only in areas where cattle had little access to pods (Impson *et al*. 1999, Zachariades *et al*. 2011). In areas where cattle were free to graze, the percentage seed damage only reached as high as 49%, as opposed to the 92% reported for areas where cattle were excluded (Impson *et al*. 1999). Despite these results, the South African mesquite programme is probably the most successful biocontrol programme worldwide to have deployed bruchids. *Bruchidius sahlbergi*, which was released in Australia for the control of *Acacia nilotica* subsp. *indica* (Mimosaceae), caused a maximum of 65% seed damage and was not considered to be particularly effective (Radford *et al*. 2001).

Sulcobruchus subsuturalis was released in 1999 for the control of *Caesalpinia decapetala* in South Africa (Coetzer & Neser 1999). However, the beetle was released in low numbers and as a consequence of this and excessive egg predation resulted in it not persisting at most release sites (Byrne *et al*. 2011). *Penthobruchus germaini* was similarly ineffective against

Parkinsonia aculeata in Australia, which was attributed to low egg densities, and it was suggested that even in the absence of the recorded egg parasitism, the required egg densities were beyond the capabilities of the beetle (van Klinken & Flack 2008).

Two bruchids, *Acanthoscelides puniceus* (Johnson) and *Acanthoscelides quadridentatus* (Schaeffer) were released for the biocontrol of *Mimosa pigra* L. in Australia and Thailand, but with a negligible impact (Napompeth 1992, Flanagan & Julien 2002). These beetles were reported to spread naturally to several other countries in Southeast Asia (Napompeth 1992, see Appendix 1), exemplifying the dispersal capabilities of bruchids. While beneficial from a biocontrol perspective, this could contribute to fears about the non-targets effects of biological control (see below). Although bruchids are mostly successful at establishing (Appendix 1), some five species (33% of those released) have failed to establish at all. These include all three species that were released against *Hyptis suaveolens* (L.) Poit. (Lamiaceae) in Australia as well as one species released against *Parkinsonia aculeata* in Australia and another released against *Prosopis* species in both Australia and South Africa (Impson *et al.* 1999, Julien *et al*. 2012, van Klinken 2012, van Klinken & Heard 2012).

4.2.2 Limitations of seed beetles

Truly successful biological control projects against invasive plants are relatively few, and often involve weeds that occur in smaller populations and where biological control interventions were initiated at an early stage of the plant's invasion (Mack & Lonsdale 2002). Potential problems with weed biological control include conflict of interest situations, where the plant is economically important; the time taken not only to set up operations, but also for them to be effective; the risk of non-target effects and; the relatively low rates of success (Markin *et al*. 1992). Also, demonstrating the degree of success of seed-feeding agents is more difficult than for agents that attack vegetative tissues (Impson *et al*. 2001). In this regard, with the possible exception of the South African mesquite programme (see above), bruchids have not been shown to deliver any major successes in weed biocontrol programmes (see Appendix 1 and references therein). A number of factors have been highlighted as potential reasons for the apparent limitations of bruchid beetles.

Mortality of the immature stages often has major consequences for biological control agents. In the case of bruchid agents, the egg and larval/pupal stages have been

demonstrated to be affected by predation, parasitism or unknown (largely abiotic) factors, which in combination are able to reduce agent populations (e.g. Coetzer & Hoffmann 1997, van Klinken & Flack 2008, Egli & Olckers 2012, Ramanand & Olckers 2013). The egg stage of bruchid beetles is particularly vulnerable to all of these mortality factors (e.g. van Klinken 2005, van Klinken & Flack 2008, Byrne *et al*. 2011) and is largely influenced by whether or not the beetles are able to conceal their eggs. For example, *N. arizonensis* does not conceal its eggs on mesquite pods and is thus more susceptible to egg parasitoids than *A. prosopis* which does conceal its eggs (Coetzer & Hoffmann 1997). *Acanthoscelides macrophthalmus* has limited opportunities for egg concealment on the smooth pods of *L. leucocephala* and suffers egg mortality of 30-50%, roughly 50% of which was the result of egg parasitism (Ramanand & Olckers 2013). At these levels, egg parasitism would cause enough damage to negatively affect beetle populations. High levels of egg parasitism on bruchid beetles has been recorded in other studies, reaching 70.5% on *P. germaini* in Australia, and 70-80% on *N. arizonensis* in South Africa (Coetzer & Hoffmann 1997, van Klinken 2005). All of the aforementioned studies describe these levels of parasitism as being detrimental to bruchid populations and damaging to biological control operations.

Similarly, in Australia, where bruchids were introduced for the biocontrol of *P. aculeata*, both the egg and larval/pupal stages were targeted by parasitoids, with 69% of eggs and 22% of larvae/pupae attacked (Cochard & Jackes 2005). Parasitism of the endophagous immature stages (larvae/pupae) generally seems to be lower than that of the egg stage, but may still be a significant source of mortality. In the case of *A. macrophthalmus*, average rates of 15% (up to 30%) larval/pupal parasitism were considerably higher than reported in other studies (see Chapter 3). It is most likely the ease of locating the eggs on the pod surface that makes them more prone to parasitism than the larval stages (Coetzer & Hoffmann 1997). The egg parasitism rates recorded by Ramanand & Olckers (2013) confirm this trend, as they are higher than the rates of larval parasitism that were reported for *A. macrophthalmus* (see Chapter 3).

Temporal asynchrony (i.e. varying rates of seed availability) has been highlighted as a strategy employed by plants (e.g. *L. leucocephala* in Australia) to reduce the effects of seed predators (Raghu *et al*. 2005). Bruchid numbers are heavily dependent on seed and seed pod availability and often decline once pods are dropped from the trees or dehisce and

release their seeds onto the ground (Radford *et al*. 2001). When the pods remained on the trees for longer, bruchid populations were able to build up and fewer seeds escaped predation (Cochard & Jackes 2005, Raghu *et al*. 2005). Also, bruchids are largely multivoltine and presumed to be less effective than univoltine seed feeders at tracking seed resources during the year (van Klinken & Flack 2008). Variable seed availability and an inability to track seed resources is generally considered to be more important than immature stage mortality in limiting the levels of seed damage (van Klinken & Flack 2008). However, both the inability of *A. macrophthalmus* to track seed resources (see Chapter 2) and larval parasitism (see Chapter 3) may play a role in limiting its efficacy.

Seeds in the canopy and on the soil surface are at risk of being eaten by a range of granivorous vertebrates, including livestock, foraging rodents and birds (Impson *et al*. 1999). In some instances, utilization of the seed pods by cattle has been implicated in the low damage levels caused by bruchid beetles (Impson *et al*. 1999, Radford *et al*. 2001, Zachariades *et al*. 2011). Livestock consumption of mesquite seeds containing bruchid larvae/pupae kills the immature stages, thus reducing the beetles' population densities and effectiveness, but also exacerbates mesquite infestations because the scarified seeds are widely dispersed in vertebrate dung where they germinate more readily (Impson *et al*. 1999, Coetzer & Hoffmann 1997, Zachariades *et al*. 2011). The impact of vertebrate consumption on populations of *A. macrophthalmus* has not been determined but may well be similar because the pods of *L. leucocephala* are also consumed by livestock and rodents (Olckers 2011).

4.2.3 Negative aspects

Bruchids have spread to several countries where they were not deliberately introduced and these include both biocontrol agents (Napompeth 1992, Tuda *et al*. 2009) and pest species (Tuda 2007, Beneen & Roques 2010). Bruchids that are more likely to feature in inadvertent introductions are those that are associated with economically important legumes that are cultivated throughout the world (Southgate 1978, Tuda 2007). In particular, *L. leucocephala* is not universally invasive and is an important agricultural plant in many countries, so introductions of potentially harmful insects like *A. macrophthalmus* are problematic (Tuda 2007). Indeed, *A. macrophthalmus* has been accidently introduced into many countries

including Australia (Raghu *et al*. 2005), Hawaii (Kingsolver 2004), West Africa (Delobel & Johnson 1998), southern Asia (Tuda *et al*. 2009) and Europe (Vassiliou & Papadoulis 2007) by means of contaminated seed that was most likely introduced from Central America. In southern Asia, *A. macrophthalmus* has been associated with the seeds of other leguminous species (Tuda *et al*. 2009, 2013) fuelling concerns about non-target effects of bruchids. The best known example of non-target effects is *Bruchidius villosus* (F.), which was tested and released for the control of *Cytisus scoparius* L. Link (Syrett & O'Donnell 1987) but which was later found to attack a non-target exotic legume in New Zealand (Sheppard *et al*. 2006). Fortunately, this study was unable to provide any evidence of *A. macrophthalmus* having non-target effects involving closely related South African legumes (see below).

4.3 *Acanthoscelides macrophthalmus* as a biological control agent

Considering the low success rates of other biological control projects involving bruchid agents, it is thus not surprising that this also appears to be true of *A. macrophthalmus*. Released in 1999, its purpose was to reduce the invasiveness of *L. leucocephala*, by inflicting high levels of seed damage (Olckers 2011). Low levels of seed damage recorded in the pilot study (Sharratt & Olckers 2012) had hinted that the beetle was not capable of complete control of *L. leucocephala*. While it is capable of damaging a high percentage of seeds, its levels of damage were largely erratic, varying greatly between sites and months of the year (Sharratt & Olckers 2012), and it never achieved the 95% level of seed damage that is required to successfully control invasive trees with high seed output (Hoffmann and Moran 1998). While seed availability of the leucaena populations monitored in this study fluctuated during the year, with low seed availability consistently recorded over a 3-month period, the inversely density-dependent relationship between seed damage and seed availability (Chapter 2) was indicative of the beetle's inability to successfully track seed densities. The results of this study were thus consistent with those of the pilot study and confirmed that *A. macrophthalmus* is not causing the required levels of damage.

Leucaena populations in Australia also experienced low numbers of *A. macrophthalmus* during periods of high seed production, allowing many seeds to escape predation and accumulate in the seed bank (Raghu *et al*. 2005). This was evident in this study, where the

high numbers of undamaged seeds found on the soil surface (Chapter 2) showed the degree to which seeds are escaping bruchid damage. The very low levels of damage to soil-borne seeds supports the contention that loose seeds are less attractive to the beetles for oviposition than seeds that are still contained in their pods (Egli & Olckers 2012). Although larval parasitism of *A. macrophthalmus* was considered to be very low (around 1%) in Australia (Raghu *et al*. 2005), the higher levels reported during this study (see above) and the density-dependent relationship between parasitoid numbers and seed infestation (Chapter 3) suggest that mortality of the larval/pupal stages is a contributing factor to the beetle's poor performance.

While *A. macrophthalmus* did not meet the expectations for seed damage, this study produced no evidence of undesirable non-target effects. There are a few species within the genus *Acanthoscelides* that are known pests of crop plants, and *A. macrophthalmus* has been reported to attack a non-target exotic plant from the family Fabaceae in Taiwan (Tuda *et al*. 2009), with more recent reports of isolated attacks of cultivated pigeon peas (*Cajanus* species) in Southeast Asia (Tuda *et al*. 2013). This led to concerns about the possibility of the beetle expanding its host range to native acacias in South Africa, which was fortunately not verified. These results thus support the results of earlier laboratory host-specificity tests (Shoba & Olckers 2010) that demonstrated that *A. macrophthalmus* does not utilize native *Acacia* species as hosts.

4.4 Potential of seed-feeding agents

Damage levels that are required to destroy invasive plant populations are often beyond the capabilities of many agents that attack vegetative tissues; however, seed feeders are more likely to affect the rates of invasion of such plants (van Klinken *et al*. 2008). The invasion rate is the speed at which the plant disperses to new sites, and is more strongly affected by seed predation than is plant population density, particularly at the levels of damage currently achieved by some seed predators (van Klinken *et al*. 2008). Seed feeders are thus considerably more effective at limiting the spread of the invader, as opposed to reducing the size of existing plant populations by affecting seedling recruitment (van Klinken *et al*. 2008). Similarly, in the case of *L. leucocephala*, slowing of the plant's invasion rate is a more

realistic outcome than a reduction in plant population densities. This will, however, depend on a number of factors including how many seeds are produced by leucaena populations, how many are destroyed by the beetle and how effectively they are dispersed, as "poor dispersers" require much lower levels of seed damage to slow invasion than do "good dispersers" (Paynter *et al*. 1996, van Klinken *et al*. 2008). In such situations, "good dispersers" are species that disperse their seeds over long distances, while "poor dispersers" are those that rely on short-distance dispersal, with seeds mostly accumulating under the parent plants. Although *L. leucocephala* is capable of long-distance seed dispersal (e.g. via water and animals), its dehiscent pods ensure mainly short-distance dispersal compared to species where the seeds are retained in their pods and are dispersed over longer distances by foraging vertebrates.

Given the track record of bruchid biocontrol agents (Appendix 1), seed damage is unlikely to reach levels where they can achieve complete control of plants that produce high numbers of seeds, largely because of the seeds in the seed bank that have escaped damage and are still capable of germinating. However, the damage caused is not completely ineffectual (Impson *et al*. 2001) because of a reduction in the probability of long-distance seed dispersal (van Klinken *et al*. 2008). For example, although *P. germaini* inflicts levels of seed damage that are too low to cause any significant reduction in populations of *P. aculeata* in Australia, it may slow down the weed's rate of spread (Cochard & Jackes 2005). Although it has not achieved the desired levels of damage, it may be worthwhile to determine whether, and how much, *A. macrophthalmus* has slowed the rate of spread of leucaena in South Africa.

In the case of *A. macrophthalmus* on *L. leucocephala*, a single agent was deployed against the target plant. Biological control initiatives involving more than one agent species are often more successful than those involving a single species, because of either additive or interactive, but seldom competitive, effects (Denoth *et al*. 2002). Agents often work better in combination, as shown by the three weevil species (a flowerbud-feeder, seed-feeder and stem-borer) that were released for the control of *Sesbania punicea* Cav.) Benth. (Fabaceae) in South Africa (Hoffmann & Moran 1998). Populations of *S. punicea* with two or more weevil species were less dense than populations with only one weevil species. One agent working on its own did not have a significant impact on the plant population, but the addition of a second agent reduced the seed set by almost 100%, resulting in a reduction in

plant numbers (Hoffmann & Moran 1998). Control of *Hakea sericea* Schrad. & J.C.Wendl (Proteaceae) in South Africa was improved by the combined impact of the weevil *Erytenna consputa* Pascoe (Coleoptera: Curculionidae) and the moth *Carposina autologa* Meyrick (Lepidoptera: Carposinidae), which destroy the immature (green) and mature seeds respectively (Gordon & Fourie 2011). Also, *Acanthoscelides puniceus* that was released to control *M. pigra* in Australia, only damaged 1% of the seeds, but when combined with other seed feeders it contributed to 20% of seed damage (Flanagan & Julien 2002). Consequently, the addition of other seed-feeding or seed-reducing agents to the *L. leucocephala* system may result in a more significant contribution from *A. macrophthalmus*.

4.5 Conclusion

Despite their potential, seed-feeding bruchid beetles have consistently been demonstrated to be ineffectual biological control agents in terms of complete control. With one possible exception, there are no instances of major success when using bruchids to control invasive alien plants and *A. macrophthalmus* is no exception to this general rule, for reasons discussed previously. Although the impact of *A. macrophthalmus* appears to be negligible, it may play a role, albeit minor, in limiting the rate of spread of *L. leucocephala* populations. This may be important considering that *L. leucocephala* has not yet spread to the extent of other leguminous invaders in South Africa, presumably because of sub-optimal climatic conditions (Olckers 2011). Also, low levels of seed damage may become more important by reducing the costs of follow-up operations when weed populations are periodically cleared, as occurs in South Africa following the advent of the 'Working for Water' Programme (see Moran *et al*. 2004). Furthermore, reduced interest in the use of *L. leucocephala* as an agroforestry plant in Africa and elsewhere in the world (see Olckers 2011) suggests that the introduction of additional agents may be a possibility. In particular, the release of an agent that attacks the immature (green) pods of *L. leucocephala* could create shortages of ripe pods which, given the beetle's inversely density-dependent relationship with seed availability, could substantially enhance the impact of *A. macrophthalmus* in South Africa.

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APPENDIX

List of seed beetles (Chrysomelidae: Bruchinae) that were considered as biological control agents against invasive seed-producing trees and shrubs around the world, including an assessment of project outcomes.

Plant species Origin *Bruchid species*	Country	Establishment	Damage inflicted [a]	Degree of control [a]	References
		ANACARDIACEAE			
***Schinus terebinthifolius* Raddi** South America					
Lithraeus atronotatus (Pic) [b]	Hawaii	Established	Trivial	Negligible	1, 2, 3, 4, 5, 6, 7
		FABACEAE			
***Caesalpinia decapetala* (Roth) Alston** Asia					
Spermophagus sp.	South Africa	Rejected	N/A	N/A	8
Sulcobruchus subsuturalis (Pic) [c]	South Africa	Established	Trivial	Negligible	8, 9, 10
***Leucaena leucocephala* (Lam) de Wit** Central America					
Acanthoscelides macrophthalmus (Schaeffer)	South Africa	Established	Trivial	Negligible	11, 12

***Prosopis* spp.**					
North & Central America					
Algarobius bottimeri Kingsolver	South Africa	Not established [d]	N/A	N/A	13
	Australia	Not established	N/A	N/A	14, 15, 16
Algarobius prosopis (Le Conte)	South Africa	Established	Moderate	Negligible	13, 17
	Australia	Established	Trivial	Negligible	14, 15, 16
Mimosestes protractus (Horn)	South Africa	Rejected	N/A	N/A	13, 18
Neltumius arizonensis (Schaeffer)	South Africa	Established	Trivial	Negligible	13, 17, 18
***Cytisus scoparius* L. Link**					
Europe					
Bruchidius villosus (Fabricius)	Australia	Established	Trivial	Negligible	4, 19
	New Zealand [e]	Established	Moderate	Negligible	19, 20, 21, 22
***Parkinsonia aculeata* L.**					
Central & South America					
Mimosestes ulkei (Horn)	Australia	Not established [d]	N/A	N/A	23, 24, 25, 26
Penthobruchus germaini (Pic)	Australia	Established	Trivial	Negligible	23, 24, 25,26, 27, 28
***Mimosa pigra* L.**					
Central & South America					
Acanthoscelides puniceus Johnson	Australia	Established	Trivial	Negligible	29, 30, 31, 32
	Thailand	Established	Trivial	Negligible	4, 33, 34
	Vietnam	Established	Unknown	Under assessment	4
	Myanmar [f]	Established	Unknown	Unknown	4, 33
	Malaysia [f]	Established	Trivial	Negligible	4, 33
	Indonesia [g]	Established	Unknown	Unknown	4, 33
	Laos [g]	Established	Unknown	Unknown	4, 33

	Singapore [g]	Established	Unknown	Unknown	4, 33
Acanthoscelides quadridentatus (Schaeffer)	Australia	No longer found	N/A	N/A	29, 30, 31, 32
	Thailand	Established	Trivial	Negligible	4, 33, 34
	Vietnam	Established	Unknown	Under assessment	4
	Myanmar [f]	Established	Unknown	Unknown	4, 33
	Malaysia [g]	Established	Unknown	Unknown	4, 33
	Indonesia [g]	Established	Unknown	Unknown	4, 33
	Laos [g]	Established	Unknown	Unknown	4, 33
	Singapore [g]	Established	Unknown	Unknown	4, 33
		LAMIACEAE			
***Hyptis suaveolens* (L.) Poit.** Central & South America					
Meibomeus sp.	Australia	Not established	N/A	N/A	35
Acanthoscelides ramirezi Johnson	Australia	Not established	N/A	N/A	35
Sennius rufomaculatus (Motschulsky)	Australia	Not established	N/A	N/A	35
		MALVACEAE			
***Sida* sp.** Central & South America					
Acanthoscelides brevipes (Sharp)	Australia	Rejected	N/A	N/A	36
		MIMOSACEAE			
***Acacia nilotica* subsp. *indica* (Benth.) Brenan** Indian sub-continent					
Bruchidius sahlbergi Schilsky	Australia	Established	Trivial	Negligible	37, 38, 39

[a] **Definition of terms** (Reference # 40)

Damage inflicted - Determined by the percentage of seeds damaged by beetles upon emergence

- Extensive: Most seeds attacked, few survive
- Considerable: More than 50% of seeds damaged
- Moderate: Fewer than 50% of seeds damaged
- Trivial: Few seeds are damaged

Degree of control – The effectiveness of the bruchid beetle in reducing the numbers or spread of the target plant

- Complete: Bruchid has completely controlled the plant, no other control methods necessary
- Substantial: Other control methods still required, but most control accomplished by beetle
- Negligible: Beetle not shown to effective in controlling plant, still able to spread or no reduction in numbers
- Unknown: No information given on the effectiveness of the beetle
- N/A: Beetle either not established or rejected and not released
- Under assessment: studies into the effectiveness of the beetle in controlling the plant currently underway

[b] Formerly *Bruchus atronotatus*
[c] Formerly *Sulcobruchius bakeri* Kingsolver
[d] Initially established, no longer found
[e] Released, non-target plants affected
[f] Spread naturally then released
[g] Spread naturally

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